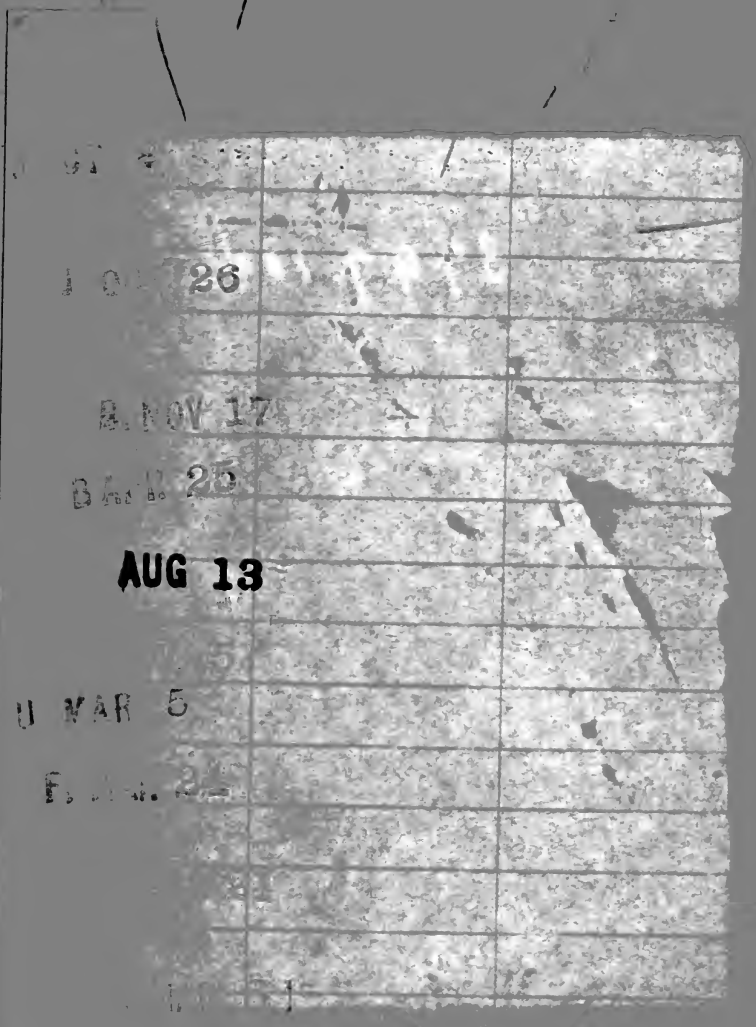
# LESSONS IN DISINFECTION AND STERILISATION

F. W. ANDREWES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# LESSONS IN DISINFECTION AND STERILISATION

## AN ELEMENTARY COURSE OF BACTERIOLOGY TOGETHER WITH A SCHEME OF PRACTICAL EXPERIMENTS ILLUSTRATING THE SUBJECT-MATTER

BY

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# PREFACE

This little book owes its origin to a practical class which I conducted in the summer vacation of 1902 for some of the nursing staff of St. Bartholomew's Hospital. It is an expansion of the lectures and practical work of which that class consisted, and I have been induced to publish it because there seems to be no elementary book which deals with the bacteriological aspects of disinfection in a systematic manner. Sterilisation and disinfection play so important a part in modern medicine, surgery, obstetrics, and public health, that their principles require to be understood by those who would practise them intelligently. They are problems in physics and chemistry applied to bacteriology, and can only be grasped from this point of view. The majority of those who are called upon to practise them have neither time nor opportunity for a complete course of bacteriological study, but it is not a difficult thing for any teacher to devise a short practical course of laboratory instruction which shall effectively teach the essential principles of disinfection. I have endeavoured in these pages to set forth the outlines of such a course. The book is written for those who know no bacteriology, but who need sufficient acquaintance

with its principles and methods to be able to understand what they are doing when they attempt to carry out processes of disinfection. I have endeavoured to keep it within a small compass by excluding all that does not immediately bear upon this one subject. I am very conscious of the imperfect manner in which I have carried out the task, but I trust that the book may be of some service not only to nurses, for whom in the first place it has been written, but also to practitioners who have no opportunity for a regular course in bacteriology, and even to those of the general public who may take an interest in such matters as are discussed.

I am responsible for the majority of the illustrations, which are, for the most part, frankly diagrammatic, though drawn from actual specimens. I have to express my thanks to Messrs. Baird and Tatlock for permission to introduce some half-dozen of their illustrations, and to Messrs. Manlove, Alliott and Co. for the figure of the Washington Lyon Steam Disinfector. It is also my pleasant duty to acknowledge the help I have received from Dr. K. J. P. Orton in discussing many chemical questions, and from Mr. George E. Gask, F.R.C.S., especially in the lesson on Surgical Disinfection, and above all, to thank Dr E. Klein, F.R.S., from whom I received my teaching in bacteriology, not only for the kindly letter subjoined, but for much wise counsel and encouragement in the preparation of the book.

F. W. ANDREWES.

*June* 1903.

St. Bartholomew's Hospital, E.C.

My dear Andrewes,

I have read these pages with the greatest interest. They appear to me to offer in a small compass and in simple and concise description all that is known and worth knowing of the why and wherefore of "Disinfection."

Every nurse and every person who is brought in contact with the sick-room will from this book be able to gather a most useful, correct and intelligible account of the manner of preventing contagion and of neutralising and destroying contagia.

Not only nurses, but I think to an equal degree medical practitioners, will be able to derive greater benefit from your book than from many other more extensive works, encumbered by a vast amount of detail, which may be useful to the bacteriologist but is hardly required or understood by the general medical practitioner. I sincerely wish your book the best success.

Believe me, yours sincerely,

E. KLEIN.

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# LESSONS IN DISINFECTION AND STERILISATION

## I

## THE GENERAL NATURE AND CLASSIFICATION OF MICRO-ORGANISMS

**Introduction.**—There was once a learned professor who wrote a book about bacteria. He began with these words: "The infinitely little are the masters of the world." Like many another epigram, it was more brilliant than accurate, and it is growing less and less true as time goes on. For man is gradually becoming the master of the infinitely little. He has pressed some of the good sorts of bacteria into his service and forced them to do his work: he is gradually learning how to exterminate the bad sorts, or at least how to keep them within bounds and counteract their evil effects. In this book an attempt will be made to explain some of the ways in which we may fight against the bad sorts. The only method by which we can hope to get the mastery over bacteria is by learning all about them, studying their habits and distribution, and testing in

the laboratory how they can most conveniently be destroyed. These are the steps by which our present knowledge has been gained, and those who would learn how to practise disinfection intelligently must follow in the same path, lest their practice degenerate into a meaningless routine. It is therefore needful, before attempting any explanation of the principles of disinfection and sterilisation, to set forth, as simply as possible, what bacteria are, how they grow and multiply, where they chiefly abound, why some are good and some are bad, and how they can be cultivated and studied. The first four lessons will be devoted to these matters in order that the rest may be intelligible. At the end of the book there will be found a short account of certain simple experiments or demonstrations designed to illustrate the subjects which have been dealt with. They are intended to be carried out under the supervision of a competent teacher of bacteriology, and it is most earnestly advised that the learner should not be content with merely reading about things, when they can be actually seen and verified. Even an elementary course of practical work such as will here be suggested, containing nothing which approaches a proper technical training in bacteriology, will be of far greater real value than any amount of theoretical instruction. Any one who has prepared an actual culture from his own hands, and seen the bacterial colonies which arise, will have a living grasp of the importance of surgical cleanliness which can be attained in no other way. The student who has seen spores grow after prolonged boiling or treatment with powerful disinfectants will actually know from his own

experience how difficult the task of disinfection may be.

* * * * *

**The Nature of Bacteria.**—The term *micro-organism* or *microbe* is used to include a number of different forms of organic life which have in common only extreme minuteness of size. Some belong to the animal kingdom, but the great majority are vegetable in nature. At the very bottom of the vegetable kingdom are two groups of simple forms, the *Algæ* and the *Fungi*. The higher forms of these, typified respectively by seaweeds and mushrooms, are distinct enough; but the lower forms, consisting only of single cells, approach one another closely. Neither group is essentially lower in position than the other, for the most elementary forms in each are about as simple as possible. The organisms with which we have here to deal are considered to belong to the fungi.

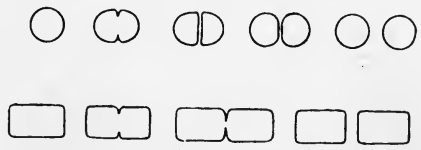

FIG. 1.—Diagram illustrating the process of simple fission, whereby one bacterium divides into two equal halves. The upper series of figures shows the process as it occurs in a coccus, the lower in a bacillus.

The lower forms of fungi are classified according to their mode of reproduction. The easiest method by which one living being can become two is by splitting into two equal halves, each of which grows to the size of the parent form. This process is called *simple fission*, and is the sole method of multiplication amongst the *Bacteria*, which are the lowest of all fungi. The formation of spores by some bacteria is not truly a mode of multiplication,

for one bacterium gives rise only to one spore; it is merely the development of a resistant "resting stage."

Another easy mode of reproduction is by *budding*. The parent form gives out a small knob, which gradually grows till it equals the parent in size, when it becomes detached. Often, before the daughter bud separates, it has itself given origin to a grand-daughter bud. The only essential difference between this and fission, is that in the latter the daughter cells are from the first equal in size, and there is no parent left; so that bacteria are said, in a sense, to have solved the problem of immortality. Budding is the mode of multiplication amongst the *Yeasts*, which further exhibit a slight advance upon the Bacteria in that they have, in addition to budding, a mode of spore formation in which several spores are formed in a single parent cell, so that it is a true method of multiplication.

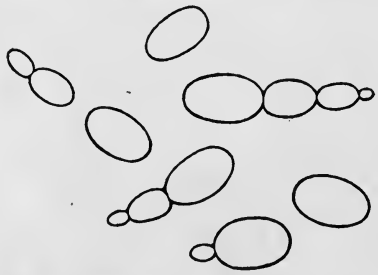

FIG. 2.—Diagram of a yeast, multiplying by the process of budding.

Neither in bacteria nor in yeasts is there anything resembling sexual reproduction. More than this, there are no special cells set aside for purposes of reproduction. Every single cell is the precise physiological counterpart of every other cell; each alike can live and multiply at its own sweet will. The arrangement is the simplest conceivable.

In the third group of fungi—the *Moulds*—the mode of reproduction is more complicated. The cells grow out into a system of branching tubes divided by trans-

verse partitions. This feltwork of tubes is called a "mycelium." From the feltwork, vertical tubes grow up into the air—the so-called "aërial hyphæ"—and upon these the special reproductive cells are situated, giving rise to clusters of spores. The patches of mould upon an old pot of jam can be seen by the naked eye to consist of a white feltwork with a bloom on the surface, which is often green or of some other colour: the bloom is made up of aërial hyphæ with their spore clusters. The spores are carried away by the air, and if they alight upon suitable soil they germinate into a new mycelium. There are some moulds in which two separate cells coalesce before giving rise to a spore, which constitutes an elementary form of sexual reproduction. The group of Moulds is therefore to be reckoned higher in the scale of fungi than the bacteria and yeasts.

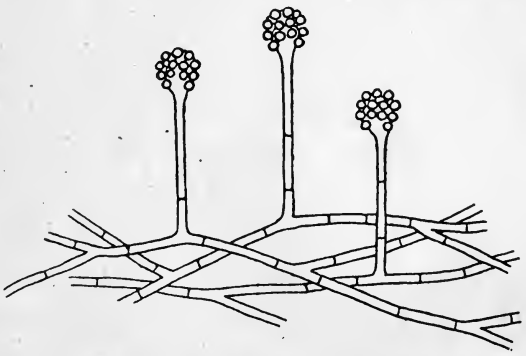

FIG. 3.—Diagram of a mould (*Aspergillus*), showing the branching mycelium from which arise the aërial threads bearing the spores.

In practical problems of disinfection not much account is taken of moulds and yeasts. They very rarely give rise to disease in man, though some forms of both are known to be capable of doing so. And in their powers of resistance to disinfecting agencies they are so inferior to bacterial spores that it may be taken for granted that what will destroy bacteria will also destroy yeasts and moulds. In the following pages, therefore, the far

more important group of bacteria will alone be considered.

**Forms of Bacteria.**—Bacteria, then, are amongst the lowest known forms of vegetable life, and they multiply by simple fission. They are unicellular; that is, each individual consists of a single cell. The individuals may, it is true, stick together into masses or threads, but each is physiologically complete and independent, and can produce innumerable progeny resembling itself.

FIG. 4.—Diagram, drawn accurately to scale, to illustrate the relative size of bacteria. The small circle represents a staphylococcus, the rod a tubercle bacillus, and the circle, in which these are enclosed, a red blood-corpuscle.

The *size* of bacteria is so minute that a special unit of measurement is used in describing it. This is the "micro-millimetre." A metre is 39.37 inches: the micro-millimetre is the millionth part of a metre, and is about one twenty-five thousandth part of an inch. The spherical bacteria (or "cocci") vary from $\frac{1}{2}$ to $1\frac{1}{2}$ micro-millimetres in diameter: most sorts measure 1 micro-millimetre or a little less. The rod-shaped bacteria (or "bacilli") are mostly from $\frac{1}{2}$ to 1 micromillimetre in thickness, and vary in length up to 3 or 4 micromillimetres or even more. Figures such as these do not, however, convey an adequate idea of the minuteness of bacteria: it is better to compare them with known objects. Most people who have used a microscope have some idea of the size of a red blood corpuscle: it would take 3200 average red corpuscles placed edge to edge

in a straight line to reach across a halfpenny. But it would take about nine of the common pus cocci (Staphylococcus pyogenes aureus) to reach across the disc of a red blood corpuscle, so that no less than 29,000 of them would be required to reach across a halfpenny. It will readily be believed that very high powers of the microscope are requisite to see such objects distinctly, but microscopes have reached such a degree of optical perfection that this presents no serious difficulty.

The *structure* of bacteria can indeed be ascertained to some extent, especially when they have been rendered more distinct by staining with suitable dyes. The cell of which each consists has a definite cell wall, enclosing a mass of protoplasm in which tiny granules are sometimes apparent. In some, the cell wall forms a swollen sheath or capsule, and in some it seems to secrete a glutinous material which sticks the individual bacteria together into a mass. Some bacteria have organs of locomotion in the form of excessively delicate filaments projecting from their surfaces. These are called "cilia" or "flagella," and by their lashing action the bacteria move about, sometimes with great activity (see Figs. 23 and 24, on pp. 147 and 150). In other bacteria no such organs are known. A further structural feature present in some, but not in all bacteria, is the formation of spores, which will be described in the next lesson.

The **classification** of bacteria presents difficulties far greater than those met with amongst the higher forms of vegetable and animal life. In classification we endeavour to group together all those individuals which sufficiently closely resemble one another into a

"species," and all those species which have certain characters in common into a "genus." The genera, again, we group together into "families," and thus we attempt to give a general view of the relationships between the different forms of life we are studying. Now, in the higher forms of vegetable life, we rely chiefly on external form and colouring in determining these relationships. We distinguish between a geranium and a larkspur by the shape of the plant, the character of the foliage and the arrangement, structure and colour of the flowers and seeds. But with such minute and elementary objects as bacteria these external characters do not carry us very far. They are employed as far as possible in classification, but we are compelled to use, in addition, other characters, such as the appearance of the bacterial mass growing on an artificial culture medium, the chemical changes produced in the medium in which the bacteria are growing and the capacity to produce disease. We have, in fact, to classify by the sum total of the characters presented by any given form, and not by its size and shape alone. And indeed certain forms present a variability in size and shape which is very surprising, and which in itself forbids our using these characters alone as a means of classification. It is, moreover, quite possible that, amongst these earliest beginnings of organic life, the limits of species are not so rigidly fixed as higher in the vegetable scale.

But, in spite of all these difficulties, we are, as a matter of fact, quite well able to distinguish between different species of bacteria in the great majority of cases, and with the advance of knowledge the diffi-

culties are growing less. In the classification of bacteria into their main groups we are able to rely on external form, and it is now necessary to explain the meaning of the chief terms used in this primary classification so far as such terms are in general use.

It is in the first place to be noted that some sorts are always spherical, or nearly so, while others grow in the form of rods. The sorts which are always spherical are distinguished as *Cocci* or *Micrococci*. Of the sorts which are rod-shaped, some are straight and others curved, or rather screw-shaped. The straight rods are commonly called *Bacilli*, the screw-shaped rods *Spirilla* or *Vibrics*. These are the three great primary groups of bacteria—the spheres, the rods, and the screws.

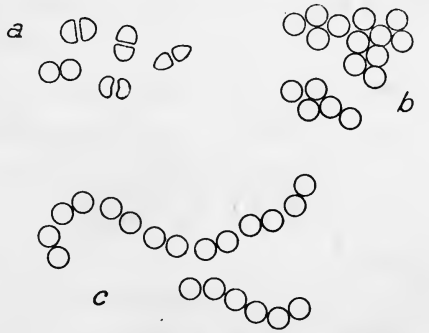


FIG. 5.—Diagram illustrating different forms of cocci. (*a*) shows diplococci of various forms; (*b*) staphylococci; (*c*) streptococci.

These great groups are further divided into smaller groups:

(1) **The Micrococci.**—The term *Coccus* is used for those bacteria which always present a spherical form. It is used in a general sense and not as the designation of any sub-group or genus. The term *Micrococcus* is sometimes used in a generic sense for cocci presenting no special mode of arrangement in chains or clusters—*e.g.*, where the cocci are all detached from one another. But most commonly cocci are arranged in a more or less definite manner; they frequently do not separate

from one another the moment division is complete, but cohere for a time, when the direction of the plane in which division has taken place determines the pattern of the grouping. Sometimes they are found always, or chiefly in pairs, and then they are designated *Diplococci.* Sometimes they are arranged in chains, long or short; and these forms are called *Streptococci.* In a few sorts, when a coccus has divided in one plane, the two resultant cocci proceed to divide in a second plane at right angles to the first, giving rise to four cocci in a square. This is the "tetrad" form. In others again, when four cocci in a square have thus arisen, each divides next time in a third plane at right angles to the two former, and so a cube of eight cocci arises. This is the origin of the cubical bales or packets which characterise the genus *Sarcina.* Lastly, it sometimes happens that the cocci cohere in irregular groups which have been likened to bunches of grapes, and the term *Staphylococcus* is applied to such. This subdivision of the cocci is, on the whole, fairly satisfactory, and seems the only one at present possible.

FIG. 6. — Diagram of the cubical packets of cocci seen in the genus *Sarcina.*

(2) **The Bacilli.**—The subdivision of these is by no means so satisfactory. They have been classified into groups according to whether or not they form spores; also according to whether or not they are motile and possess cilia, and if so, whether the cilia are arranged all round the bacillus or form a tuft at one end. Hardly any two writers are agreed as to the generic names to be applied to the different groups, and it is not proposed to give any names or definitions here. For the

present it will suffice to use the term *Bacillus* in a generic sense for all the straight rod-forms. Nevertheless it must not be supposed that the bacilli do not exhibit natural groups of the value of genera. To a large extent they do so, and in actual practice one commonly speaks of the "Coli group" or the "Diphtheria group" (or Diphtheroid group) to indicate the group of bacilli closely allied to the Bacillus coli communis or to the Bacillus diphtheriæ respectively. Some day, some one with a genius for classification will propose acceptable generic names for these groups; those which have hitherto been suggested are too cumbersome for general use.

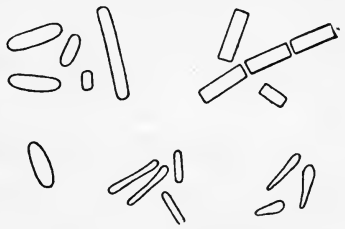

FIG. 7.—Diagram illustrating the various forms of bacilli.

(3) **The Spirilla.**—The need for subdivision here is not very great, as the group is a small one. The term *spirillum* is employed for screws with many turns. Such often break up into short "comma bacilli," each corresponding to a half turn of the screw, which are known as *vibrios*. Other terms are also sometimes used to indicate the tightness with which the screw is coiled, but they need not detain us here.

FIG. 8.—Diagram of *Spirilla* and *Vibrios*.

(4) There are certain small groups of micro-organisms commonly reckoned amongst the bacteria which have not yet been mentioned. The most important of these is the genus *Streptothrix*, because it includes the ray-fungus, which causes actinomycosis. The members of this group show an advance on the structure seen in

the true bacteria. They present a network of true branching filaments, and they further show indications that certain parts of the mycelium are set aside for reproductive purposes. These characters, with their mode of growth on culture media, bring them into some sort of relationship with the "moulds." They form, indeed, a kind of connecting link between the true moulds and the true bacteria.

## II

# THE GROWTH OF BACTERIA: THEIR REQUIREMENTS AND DISTRIBUTION IN NATURE

THE way in which Bacteria multiply has been mentioned in the preceding lesson; it is by division into two equal, or nearly equal portions; each of which rapidly attains the size of the parent organism. The rate at which, under favourable conditions, this process takes place is surprising. It can be directly watched under the microscope, and the series of changes by which one bacterium becomes two has been found to occupy about half an hour, or even less. So that in an hour there may be four where there was one, and in two hours sixteen, and in four hours two hundred and fifty-six. If the calculation be pursued it will be found that the persistent fission of a single bacterium, once every half-hour, would yield, in twenty-four hours, a progeny of billions. It is not to be supposed that this rate of multiplication is ever actually attained. It is not very likely that the daughter products of a single division proceed instantly to divide anew. And if they did, the amount of space and of food available would speedily run short, checking the increase of population through overcrowding. Nevertheless the figures are of value as illustrating the vast

potentialities for increase possessed by bacteria. If a sterile tube of broth, perfectly clear and transparent, be inoculated with the minutest possible trace of a culture of the common pus coccus—Staphylococcus pyogenes aureus—and incubated at the temperature of the body, it will be found after twenty-four hours to be densely turbid, and every drop will contain countless myriads of the cocci.

**Spore formation** is not so much a method of multiplication as the development of a resistant resting stage. True spores are not formed by cocci, but only by the rod-shaped bacteria, and only by some of these. When spore formation is about to take place, a bright spot appears in the bacillus, gradually increasing in size and distinctness. It now takes the form of a highly refractile round or oval body, clear and sharply defined, while the remainder of the bacillus shrivels and becomes indistinct, finally breaking up and disappearing, so that the spore is liberated. In some sorts of bacilli the spore is formed in the middle of the rod, in others at one end, so that a "drum-stick" form arises, but no individual bacillus forms more than one spore, and it uses itself up in the process.

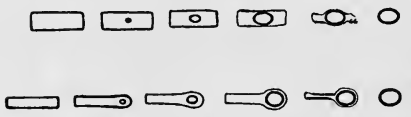

FIG. 9.—Diagram of spore formation. The upper series of figures shows the formation of central spores in the anthrax bacillus; the lower series the formation of terminal spores in the tetanus bacillus.

The object of spore formation is evident from the properties of the fully formed spore. Its distinct outline and bright appearance are due to the possession of a thick coat or capsule of a very impenetrable kind, which does not easily allow heat or injurious chemical

agents to get at the living protoplasm within. The dyes which are used in staining bacteria act readily and intensely, in a few minutes, on ordinary forms, without the application of heat. But they penetrate the resistant capsule of the spore with extreme difficulty, and in order to stain spores it is best actually to boil them in the dye for some little time. It will presently be seen that what is true of dyes is also true of disinfectants: heat and chemicals take far longer to kill spores than to kill non-sporing bacteria. The coat of the spore is equally impenetrable to moisture, so that the living protoplasm in its centre does not easily dry up and perish when the medium in which the bacillus is living becomes dried. Spores may live for years in the dried condition, whereas few non-sporing bacteria can survive more than a few months at the outside. It is evident, then, that it is a great advantage to a bacillus to form spores, for it can thus survive injurious external conditions which would have been fatal to it in its ordinary growing state. When the danger is over, the drought past, and the surroundings again suitable for active life, the spore germinates. Its thick capsule ruptures and the protoplasm within grows out into a new bacillus.

The importance of spores in all questions of disinfection is thus very great, because they are many times over as hard to kill as the growing forms of bacteria. Unless we know for certain that the infective microbe in any particular case is one which never forms spores, we have to plan the campaign as against the most resistant known forms of bacterial life. And, indeed, in order to be on the safe side, it is always well to do this, for it is possible that some species not at present

known to form spores may actually do so. Moreover, in many fevers and infective conditions the actual micro-organism is as yet undiscovered, so that we do not know whether it forms spores or not.

**The Requirements of Bacteria.**—All living things demand, for their growth and activity, certain fundamental conditions. They must have moisture, food, a suitable temperature, and other convenient physical surroundings. In the absence of these, life may indeed be maintained for awhile, but no activity or multiplication can occur. The needs of bacteria are shortly as follows:

**Moisture** is as needful to them as to other living things. The bacteria in dust and those floating in the air are in a condition of suspended animation, ready to germinate should they fall upon a suitable material, but quite incapable of growth under existing circumstances.

**Food.**—Bacteria are plants, and, as such, have the power of living on very simple food, upon which no animal could subsist. The fundamental distinction between an animal and a plant is that the plant can build up its own protoplasm out of comparatively simple mineral ingredients, while the animal has to feed on ready-made albuminous material. It is possible to make, in the laboratory, a solution of simple chemical substances containing all the essentials necessary for plant life, in which some bacteria will grow and thrive. But, amongst plants, fungi are peculiar in that they prefer to save themselves the trouble of building up their own protoplasm from the very beginning. Most bacteria like, if they can, to feed, not on simple mineral foods,

but on the more complicated food provided by organic matter. Really plants, and able, at a pinch, to build up their own albumin, they more often save time and trouble by feeding on it ready-made as animals do. The common food of nearly all bacteria, and of all with which we are here concerned, is dead or living organic matter—*i.e.*, material of animal or vegetable origin. It is not necessary to go into detail about the precise chemistry of their needs, save in one respect. Amongst the elements requisite for every form of life is oxygen. This is a gas existing in the free state in the atmosphere. The majority of bacteria like free oxygen, and flourish in its presence, but some cannot endure it, and will not grow at all except in its complete absence. These are therefore called *anaërobes*, while those that grow freely in its presence are called *aërobes*. There are a great many bacteria which can grow well under both conditions—in the absence as well as in the presence of free oxygen. Oxygen is an essential

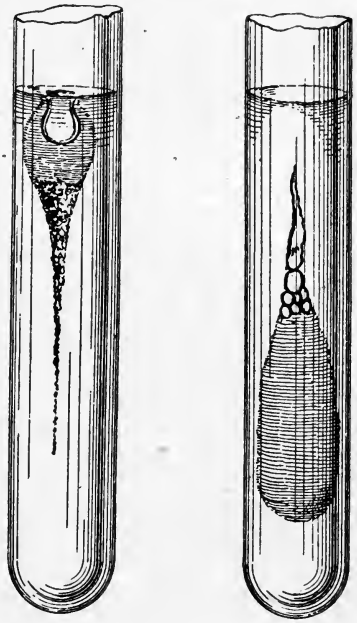


FIG. 10.—Drawing of stab cultures in gelatin illustrating the growth of an aërobic and of an anaërobic organism. In (*a*) the growth is aërobic and attains its maximum near the surface, where a cone of liquefaction has developed, with a depression containing air. In (*b*) the growth is anaërobic, and attains its maximum deep in the substance of the gelatin medium. Near the surface of the gelatin there is no growth at all. Some gas bubbles have accumulated at the upper end of the liquefied growth. (Drawn from a photograph.)

ingredient in all living protoplasm and is needful for the chemical changes which accompany the life of the simplest cell; the anaërobes require it as well as the aërobes, and they get it by chemically decomposing substances such as sugar in the medium in which they are growing. It is only in the free state that they hate it. This habit of breaking down chemical compounds in order to get their oxygen renders the anaërobes powerful agents of chemical change in the medium in which they happen to be growing. It is to anaërobes that the putrefaction of the body after death is chiefly due. A few bacteria which cause disease in man are anaërobes: the tetanus bacillus is one of the best known of these.

**Temperature** has a marked influence upon the growth of bacteria. For each particular sort there is a temperature at which it grows best, and this is called the "optimum" temperature for that species. Different sorts have different optimum temperatures, but we can roughly divide bacteria into three main groups in this respect. One group grows best at about the ordinary summer temperature of the air—say 70° F. To this group belong most of the common bacteria found in water and air; the majority of these are incapable of growth at the temperature of the human body. In the second group are a number of bacteria whose optimum is about the temperature of the human body—say 98° or 100° F. It is to this group that those bacteria belong which are capable of producing disease in man; many of them can still grow, though less vigorously, at the ordinary temperature of the air, but some cannot do this. The third group comprises a few remarkable

bacteria which grow best at very high temperatures, even at 160° F.—a temperature which would be speedily fatal to most forms of bacteria. No practical importance is at present known to attach to these curious species. In cultivating bacteria in the laboratory it suffices, for ordinary purposes, to maintain two incubators, one with a constant temperature of about 70° F. for the first group, and one with a constant temperature of about 98° F. for the second.

In addition to the temperature at which it grows best, there is, for each sort of bacterium, a temperature above which it cannot grow at all, and also a temperature below which it cannot grow at all. These are called the "maximum" and "minimum" temperatures for that species. Some species have a fairly wide range, others a more restricted one, in their temperature requirements. In all cases growth ceases long before the freezing-point of water is reached, and, if we except the bacteria in the third group just mentioned, no growth occurs above 110° F.

**Light** is the most important of the other physical conditions which influence the growth of bacteria. They love darkness rather than light. Many will grow in the light, though less vigorously than in the dark, but most sorts are killed by prolonged exposure to light, and fairly soon by the action of direct sunlight. It follows that the admission of sunlight into the sick-room is a valuable thing. Koch found that the tubercle bacillus was killed by direct sunlight in a few hours, or even less where the layer of bacilli exposed was very thin: diffuse daylight killed it in about a week.

**The Distribution of Bacteria in Nature.**—The places where bacteria naturally grow are those in which their vital requirements, as above outlined, are fulfilled. They grow wherever they get the chance. On and in moist organic matter generally, provided that it is not too strongly exposed to light, the conditions are favourable to them. The degree of moisture which they require is not large, and the amount of food necessary for such minute organisms is very small. Ordinary drinking water of good quality may contain the merest traces of organic matter, perhaps one part in a million, yet this is ample for the growth of many sorts of bacteria. No water is so pure as to be free from bacteria, and the more impure it is the larger will be their numbers. A good drinking water should not have more than 50,000 bacteria in an imperial pint, but we commonly drink waters containing five or ten times this number. Sewage is an ideal breeding-ground for bacteria, and in a pint of London sewage from the Barking outfall there may be as many as 18,000,000,000. The animal body likewise offers admirable facilities for their multiplication quite apart from any invasion of the living tissues. Our skins are covered by a layer of dead organic matter, which varies in thickness according to our habits of personal cleanliness, but is never quite absent. In this many sorts of bacteria abound, as may readily be proved by cultivation experiments, and it has been shown that their numbers steadily increase in our underclothing from the time we put it on fresh from the wash till we cast it off on Saturday night. On the inner, or mucous surfaces of the body the conditions are even more favourable for bacterial growth, on account

of their greater moisture and more equable temperature. In the mouth they are present in incredible numbers; the saliva of a normal person contains, volume for volume, three times as many bacteria as sewage from Barking Creek. This fact is unpleasant to reflect on, but can readily be verified by direct experiment (see p. 209). In the intestinal contents they are present in even larger numbers, and indeed it is probable that nowhere are the opportunities for bacterial growth so great as in the large intestine of man and animals. In the soil, bacteria abound in proportion to its degree of organic contamination. Cultivated soils which are freely manured contain enormous numbers in the surface layers, but the numbers diminish away from the surface, and a few feet down they are relatively scanty.

The distribution of bacteria does not, however, strictly correspond with the facilities for their actual growth and multiplication, for many of them can survive drying for considerable periods, and can be carried about by the air in a condition of dormant vitality. The dust of our streets and rooms contains much dried organic matter, crowded with bacteria. Street dust is partly composed of horse-dung, and there are those who spit on the pavement. Even in talking it is impossible not to eject minute particles of saliva, while every particle of skin detached from the surface of the body adds its share to the contamination of the air. It follows that wind, and everything which raises dust, plays a great part in the conveyance of bacteria. It is natural that the air in crowded centres of population should contain many more bacteria than country air. And it is said that on the summits of lofty mountains, and on the

ocean far from land, the air contains none at all. They are more numerous in warm weather than in cold, because warmth favours multiplication in their natural breeding-places. Rain exerts a marked influence in purifying the air from bacteria, for it mechanically carries them down to the ground as it falls, and snow is even more effectual.

It is important to bear in mind that bacteria are not given off to the air from moist surfaces: they only pass off when dried. In quiet breathing they do not pass out in the breath, though in talking, coughing and sneezing, minute particles of fluid are necessarily ejected, and may be carried for some distance in the air. This has been shown by a simple but effective experiment. There is an organism known as Bacillus prodigiosus, not often found in air though common in the soil: its colonies are of a vivid crimson colour, and are hence easily recognisable. It is quite harmless. The mouth is first thoroughly rinsed out with a culture of this bacillus, and the experimenter enters a quiet room in which sterile plates of a suitable culture medium are exposed in different places; he then harangues an imaginary audience for half an hour. It is found, on subsequent incubation of the plate cultivations, that he has infected them with the Bacillus prodigiosus for a considerable distance, up to twenty feet or even more. Similarly it has been proved that, in splashing experiments with sewage, the air may be infected with sewage bacteria. But quietly flowing sewage gives up none of its bacteria to the air, and sewer air has been shown to be actually more free from micro-organisms than fresh air examined at the same time. Laws, in the course of

numerous experiments, was unable to detect in the air of sewers any of the common organisms found in sewage. It is indeed a well-established fact that bacteria cannot escape into the air from fluids or moist surfaces apart from such agitation as shall disseminate particles of the fluid itself.

The actual number of bacteria found by experiment in the air is very small in comparison with the number in water. In the streets of London not more than twenty or thirty micro-organisms are present in a pint of air, and many of these are moulds and not bacteria. Often the number is much smaller than this. The reason is that they are only accidentally carried by the air; they cannot grow and multiply there.

## III

# THE CHEMICAL ACTIVITIES OF BACTERIA AND THEIR POWER OF PRODUCING DISEASE

EACH tiny speck of protoplasm constituting a bacterium lives its own life as best it can, taking the food it requires from the medium by which it is surrounded, and passing out into that medium the products of its vital activity. The effects which each individual bacterium thus produces would be infinitesimal, but, owing to the vast powers of increase they possess, the sum total of the changes produced may be very far from small. It is, indeed, clear that bacteria play a most important part in the economy of nature. The decomposition of dead animal and vegetable matter and the series of chemical changes which fit it anew for the sustenance of plant life are all shown to depend directly upon bacterial activity. In the absence of bacteria no decomposition whatever takes place in meat or other foods. The process of preserving foods by "tinning" depends simply upon the destruction of all bacteria present by heat and their subsequent exclusion by hermetical sealing. It is not even necessary to destroy the bacteria, if their activities can be annulled by a sufficiently low temperature: frozen meat is

readily delivered to us in good condition from the Antipodes.

**Putrefaction** is one of the most obvious and familiar results of bacterial action. But only a few kinds of bacteria have the power of bringing about this change in organic matter. They are common and almost universally distributed sorts, and hence the process is almost universal. The change is wrought by the splitting up of the complicated substances of which protoplasm consists into simpler and simpler chemical bodies, the final type of which is ammonia. Many of the intermediate substances produced smell very badly. The bacteria are obliged to break up the complicated bodies in order to get the food they require, and the anaërobes are much more potent agents of chemical change than the aërobes, because they have to get even their oxygen from organic matter (see experiment on p. 183). The main agent in the putrefaction of the body after death is a spore-bearing anaërobic bacillus. But when the putrefactive bacteria have done their work, a further change is needful before the products are fitted again for the food of plants. The ammonia formed requires to be oxidised into nitric acid, and this is performed by a special group of micro-organisms known as "nitrifying bacteria," abundantly present in the soil and elsewhere. These nitrifying bacteria subsist on exclusively mineral food. It is even proved that there exist bacteria which can fix the free nitrogen of the atmosphere and render it available for plant food: they live in the curious nodules which are present on the roots of leguminous plants (peas, lupins, &c.).

Apart from putrefaction there are a number of chemical changes in organic matter which are directly due to bacteria. These are commonly known as *fermentations*. One of the best known, viz., alcoholic fermentation, is due, not to a bacterium, but to a yeast. The yeast plant, growing in a solution containing sugar, splits up the sugar, in virtue of its food requirements, into alcohol, carbonic acid and other substances. Other fermentations are due to actual bacteria—for instance, the souring of milk. This is due to the lactic acid bacillus, or perhaps to several different lactic acid bacilli commonly distributed in nature. These very readily get into milk and soon bring about the transformation of the milk sugar into lactic acid, whereby the milk turns sour and curdles.

There are many bacteria which give rise to beautiful pigments, red, orange, green or blue: milk and articles of food occasionally become thus coloured owing to bacterial growth. Some can actually produce light. The phosphorescence sometimes seen in the sea is commonly of this origin; the greenish-yellow light is emitted when the water is disturbed and brought into contact with the air.

Some of the products to which bacteria give rise are poisonous to man. Bacterial activity, outside the body, may hence sometimes cause disease, if its products are subsequently swallowed. Tinned foods, which have become partially decomposed or altered by the growth of bacteria, may be highly poisonous when eaten. Ptomaine poisoning is of this nature. Ptomaines are poisonous substances analogous to the vegetable alkaloids, produced by the action of bacteria in the

putrefaction of meat, or of other forms of albuminous matter.

**The Production of Disease by Bacteria.**—Much more commonly, however, it is the bacteria themselves which gain access to the body and lead to disease by growing and multiplying there. Comparatively few bacteria have the power of doing this. In order that they should do it they must be able to grow at the temperature of the animal body, and in addition they must have the power of getting their food from the living animal tissues, and not merely from dead organic matter. In other words, they must be able to live as *parasites*. When bacteria are living on dead organic matter they are called *saprophytes*, or are said to be living "saprophytically." The distinction between the parasitic and the saprophytic mode of life is a very important one. The vast majority of bacteria are pure saprophytes and have no power whatever of attacking living tissues. Yet even these may sometimes lead to disease. Growing in food outside the body, they may give rise to poisonous substances, and they may do the same thing on the surface of the body. The multitudinous bacteria which infest the mouth and intestine derive their sustenance from the dead organic matter which lines or occupies those cavities: they are not necessarily parasitic. They are, in a sense, outside the body proper, and yet their poisonous products may be absorbed into the body and cause disease without any invasion of the actual living tissues by the bacteria. This poisoning from without, in the absence of true infection of the tissues, is called *sapræmia*. Familiar examples of the condition are seen in the fever and

other constitutional disturbance produced by the absorption of the discharges from a foul wound, or of the decomposition-products of a piece of placenta retained in the uterus after childbirth.

But in the case of some saprophytic bacteria there exists the power of invading the living tissues should opportunity occur. Healthy tissues may resist their attacks, but the tissues are not always healthy. The natural resistance of the body against infection may be lowered by mechanical injury, by shock, by chill or by anything which impairs the general health and vitality. There is a bacillus which inhabits the intestine in great abundance, known as the *Bacillus coli communis*. As a rule it lives harmlessly on the dead organic matter furnished by the intestinal contents; that is, it lives as a saprophyte. But if, from perforation of the intestine by an ulcer, its contents pass into the peritoneal cavity, this bacillus is capable of setting up an acute peritonitis. If the appendix vermiformis becomes inflamed, or is the seat of chronic catarrh, this same bacillus may enter the weakened wall of the tube, and, growing in the unhealthy tissues, may make its way through into the peritoneum and set up peritonitis. Again, there is a coccus known as the *Diplococcus pneumoniæ*, or as the "pneumococcus," which commonly inhabits the mouth and mucous membranes of the respiratory organs as a saprophyte, unable to get any foothold in the living tissues, which in the healthy condition are a barrier to its ingress. Yet, if the vital resistance be lowered by a sudden chill, the barrier may be broken down, the pneumococci may be enabled to invade the lung and set up the acute inflammation known as pneumonia. We must thus

distinguish between the purely saprophytic bacteria and this second group, which, habitually living upon dead organic matter, can yet attack living organic matter when favourable conditions arise. Such bacteria are sometimes called *facultative parasites*, and they are of much importance, especially to the surgeon; for, owing to them, every one carries about, in and on his own person, possible means of his own infection. In yet another group of bacteria the parasitic habit is more firmly established. There are many which always get their food from the living animal tissues and cannot live in any other way. They may indeed possess sufficient resistance to maintain their life for a time outside the animal body, but they cannot grow and multiply there. So far as we know, the tubercle bacillus is such an organism. In the tissues of a susceptible animal it can grow well, setting up the disease known as tuberculosis, but there is no proof that it ever leads an active independent existence, or can derive its nourishment from any source other than the living body. We can, it is true, train it to grow in test-tubes on specially prepared culture media, but it is not easy to do so, and in the case of the allied leprosy bacillus even this has not been done. Bacteria which can only grow in the living body are called *strict parasites*, and there are a number of human diseases due to such organisms. In addition to the strict parasites there are other bacteria which, preferring a parasitic existence, and attaining their most luxuriant growth within the living body, can yet manage to subsist outside the body upon not-living organic matter, and this double habit of life confers on them a great power of spreading disease. Two familiar instances

of bacteria which can live outside as well as inside the body, though preferring the latter, are found in the typhoid bacillus, and the vibrio which is the cause of Asiatic cholera.

A little reflection on the foregoing facts will show that the habit of life of the particular bacterium which causes any given disease must exert a great influence upon the way in which that disease spreads, and must correspondingly affect the plan of campaign in our attempts to stamp it out. A few examples will illustrate this.

(1) Suppose the pathogenic organism to be a strict parasite, and one of very little resistance, so that it can only maintain life for a short time outside the body. Such an organism is the *gonococcus*, known to be the infective agent in gonorrhœa. It is not only a strict parasite, but one confined to the human species; it cannot grow in or on the bodies of lower animals. The gonococcus is therefore practically found only in cases of the disease in human beings, and gonorrhœa can spread from one person to another only by direct contact. The only indirect way in which the disease can be conveyed is where an article, such as a sponge or a handkerchief, soiled with fresh gonorrhœal discharges, is used by a healthy person. The eyes have thus been infected and gonorrhœal ophthalmia has resulted. Such an article, however, on drying, would soon lose its virulence, and it is customary to regard gonorrhœa as a typical instance of a directly contagious disease. The range of action of the gonococcus, viewed as an infective agent, is thus an extremely limited one, and it would be correspondingly easy to stamp out the disease by suitable measures of isolation and disinfection.

(2) Again, let the infective agent be a strict parasite, but one capable of considerable resistance outside the body, able to withstand drying for months or even years without losing its vitality. The *anthrax bacillus* is such an organism. There is some evidence that it may, under favourable circumstances, multiply outside the animal body, though its power of doing so is limited, but its spores can remain dormant in the soil for years. The *tubercle bacillus* is another. It is not clear that it forms spores, but it is certain that dried tubercle bacilli remain living and virulent for many months. This persistence of vitality, for long periods outside the body, greatly increases the range of action of such bacteria as infecting agents, for in addition to direct, it confers vast opportunities for indirect infection. An animal dead of anthrax may infect a pasture for years, so that other animals grazing there are liable to be attacked. A consumptive, spitting on the pavement, furnishes material which, as dried dust, may infect many a susceptible individual after months. It follows that the measures required to combat such diseases are far more complex than in the case of a strict parasite with little resistance. It is not a difficult thing to employ disinfection in all cases of the disease coming under observation, but it is an impossible task to disinfect the whole face of Nature. We are forced to be content with very partial measures. And when, as happens to be the case in anthrax and tubercle, the disease is one common to man and many of the lower animals, so much the more overwhelming are the difficulties against which we have to contend.

(3) There is a third group of diseases in which the

infecting agent is not a strict parasite, but an organism capable not merely of maintaining its vitality, but of growing and multiplying as a saprophyte outside the body. The typhoid bacillus and the cholera vibrio have been already mentioned as cases in point. Water or soil which has been polluted by the excreta of typhoid or cholera cases may serve as the breeding-ground for the specific bacteria. It is fortunately true that the constitutions of these organisms are not sufficiently hardy to enable them to go on growing indefinitely outside the body. In polluted water or soil they come into competition with much hardier and more vigorous saprophytes, and are ultimately elbowed out of existence. But they can live and multiply long enough to cause wholesale contamination of wells and water-supplies, so that a single case of typhoid may, if the conditions are favourable, serve as the starting-point of a serious and widespread epidemic. The capacity for growing as a saprophyte thus confers upon a disease-producing bacterium a greatly increased power for evil.

There are some bacteria which, having set up disease by their growth in the living tissues, can still remain living as saprophytes on the mucous surfaces of one or other of the body cavities after the attack of disease has ceased. In the course of an attack of diphtheria the patient, if he recover, becomes more or less immune against the diphtheria poison, at least for a time. The diphtheria bacilli can no longer live parasitically in his tissues, but there is nothing to prevent their continuing to live as saprophytes in his throat. In certain cases they actually do so for months, and such cases, even though they are in good health, are dangerous sources

of infection to others. There is good reason for believing that, after an attack of typhoid fever, the specific bacilli may similarly go on living for many months as saprophytes in the intestine.

The foregoing illustrations must serve to indicate the extreme importance of an accurate knowledge of the habits of life of the different disease-producing bacteria if an intelligent war is to be waged against the diseases they produce. If that war could be waged only by means of sterilisation and disinfection, the outlook would indeed be gloomy, for the foe is so numerous, so ubiquitous, so resourceful, and often so resistant, that the prospect of its extermination seems hopeless. Fortunately, however, there is another side to the matter, though one which can only be briefly mentioned here.

We speak of bacteria as the "cause" of infective diseases, and so in a sense they are. When one strikes a match and lights the drawing-room fire, the match is, in a sense, the cause of the fire. But the fuel must have been dry and properly laid or no fire would result. So the tubercle bacillus is the cause of tuberculosis in the sense that the disease could not be set up without it. But there must be a susceptible soil on which it can grow, or no disease will occur. The ordinary healthy person does not present such a soil, but there are certain persons and certain families that do so. Even those not naturally predisposed may become so by living under unhealthy conditions. Overcrowding, deficient ventilation, lack of fresh air and sunshine, poor food—all these are conditions which help to render the body a suitable soil for the growth of the tubercle

bacillus by lowering its vital resistance. These, too, are "causes" of tuberculosis, just as essential as the tubercle bacillus. We call them *predisposing causes*, and the bacillus the *exciting cause*. What is true of tubercle is true of other infective diseases. In every case it is not only a question of the advent of the infecting-agent, but also of the susceptibility of the person infected. It is a matter of common knowledge that individuals vary widely in their susceptibility to infectious diseases; but in a broad sense it is true that the healthy person, living under healthy conditions, is less susceptible, and the unhealthy person, living under unhealthy conditions, is more susceptible. It is therefore possible to wage war against infectious disease from a different standpoint—to trouble less about the bacteria and more about the health, and hence the vital resistance of the population. Practical experience has shown that the mortality from tuberculosis can be considerably reduced by improving the hygienic conditions under which people live—by diminishing overcrowding, by improving ventilation, and letting in fresh air and sunlight to their dwellings, and this without troubling our heads about the tubercle bacillus at all. The modern treatment of phthisis in sanatoria consists essentially in placing the patient under the most ideal hygienic conditions, and thus improving his powers of vital resistance until his tissues get the better of the bacilli which have invaded them.

A few words must be said in conclusion as to how the parasitic bacteria cause disease when they invade the body. They do so mainly by poisoning the tissues. The actual protoplasm of which some bacteria consist

is directly poisonous to the animal body. This is proved by the fact that even dead bacteria are in some cases able to set up disease when injected experimentally in animals. Apart from this, many bacteria secrete virulent poisons which are soluble and pass out from their bodies into the surrounding medium. Broth cultures of the tetanus bacillus, filtered through porcelain, so as to remove all the bacilli and leave only their soluble products, are found to contain a most intense chemical poison, capable, when injected into an animal, of setting up all the phenomena of fatal tetanus. The exact chemical nature of these poisons is not known, and we speak of them somewhat vaguely as "toxins." The symptoms of poisoning due to their absorption into the system are often spoken of as "bacterial intoxication."

It has already been stated that disease may arise from the taking into the body of bacterial poisons produced by saprophytic bacteria in food or in the cavity of the intestine. Naturally, the poisons are even more readily absorbed when the bacteria are growing as parasites within the actual tissues. Nor is it always necessary that the bacteria should spread widely within the body. A distinction is made between "local" and "general" infection. Good examples of local infection are furnished by tetanus and diphtheria. In these diseases the bacteria habitually remain localised at or near the point of infection, multiplying in the tissues and producing their specific poisons. The symptoms of disease are due to the absorption into the circulation of these poisons—*i.e.*, there is local infection but general poisoning. In other cases, the bacteria

themselves pass into the circulation, grow and multiply in the blood and are conveyed all over the body. This is general infection, and the condition is known as "septicæmia" when the bacteria are actually growing in the blood. Many bacteria can cause septicæmia, though the commonest is the *Streptococcus pyogenes*. Sometimes, as in typhoid fever and acute general tuberculosis, the blood serves as the channel of dissemination, though the bacilli do not actually grow and multiply in it. This is to be distinguished from true septicæmia.

The poisons of different bacteria act in different ways and upon different tissues. Some simply kill the tissues in which the bacteria grow and produce local tissue death—known as "gangrene" or "necrosis." Some irritate the tissues and cause an accumulation of white blood corpuscles (leucocytes), giving rise to suppuration or other form of local inflammation. Some, when absorbed into the circulation, exert their deleterious action upon particular tissue-cells with which they seem to have a special chemical affinity. Thus the tetanus poison exerts its action upon the cells of the central nervous system.

It would lead us too far from the subject of this book to discuss the nature of immunity and of resistance against bacteria. It must suffice to state that the body possesses the power of forming substances antagonistic to bacteria and their poisons. The substances which act as antidotes to their poisons are called "antitoxins." Those which antagonise the bacteria themselves are substances capable of breaking up and destroying the bodies of the bacteria. Sometimes antagonistic sub-

stances are naturally present in the blood and tissues, and this constitutes "natural immunity." Their production is also evoked by the presence of the bacteria. Thus, after an infection from which recovery has taken place, anti-bodies are found to have been formed, and this constitutes "acquired immunity."

# IV

## THE ARTIFICIAL CULTIVATION OF BACTERIA

Not all bacteria can be induced to thrive in captivity. There are some very strict parasites which have never yet been cultivated, because the conditions to which they are accustomed in the living body cannot be closely enough reproduced outside it. But with care and attention many parasites can be induced to grow in test-tubes, some readily, others with difficulty and only for a short time. The exact requirements of each species have to be found out by repeated trial till the proper soil and temperature are discovered. The majority of saprophytes grow vigorously under artificial conditions. It is of great advantage to be able to grow a species in the laboratory; indeed, the greater part of our knowledge of bacteria has been thus acquired. Of the sorts we cannot cultivate we know very little.

The principles involved in cultivating bacteria are similar to those involved in cultivating other vegetables. When we wish to grow mustard and cress in a window-box we provide a suitable soil, taking care that it is free from weeds, plant the seeds therein, water them, and see that the conditions as to temperature and light are suited to the known requirements of mustard and cress.

The details of cultivation are different in the case of bacteria, but the principles are the same. It is needful to provide a suitable soil for their growth, one, that is, which shall meet their food requirements. The soil must be free from "weeds"—that is, from germs other than those we wish to cultivate. It is requisite to sterilise it first, and then to prevent the subsequent access of other bacteria. The bacteria to be cultivated are then planted in or on the soil, which must be prevented from drying up, and must be maintained at a suitable temperature, and otherwise kept under conditions known to be favourable to the growth of the species in question.

The earlier bacteriologists used only fluid media for the cultivation of bacteria. Troubles arose from this, for if two sorts or more chanced to be growing in the same tube, they naturally were so intermingled that it was a matter of great difficulty to separate them. The introduction of solid culture media was a great advance. Bacteria cannot move about on a solid surface; they have to grow where they lodge. If we dilute a fluid containing different sorts of bacteria so that the individuals are sufficiently far apart, and then spread a drop of it over the surface of a solid culture medium, the individual bacteria will lodge at intervals on the surface and each will grow and multiply where it lodges. By the time it has produced a progeny of a few millions, which is usually in a day or two, the mass will be visible to the naked eye, and is now called a *colony*. Each colony will consist of one single kind of bacterium, being the progeny of a single individual, and as a rule it is possible to distinguish between the colonies of

different kinds, because, seen in the mass, they differ even to the naked eye, in size, shape, colour and consistency. From the different colonies *sub-cultures* may now be made into fresh sterile culture tubes, and thus we may readily obtain, from the original mixture, *pure cultures*—(that is, cultures consisting of one single species of bacterium) of the different sorts present. The characters of the growth on solid media are also a valuable aid in distinguishing between different species, and it is often possible to determine at a glance whether a culture is pure or contaminated by the presence of foreign organisms.

Of soils or *culture media* for the growth of bacteria we have a large choice, some suited to one sort, others to another. Many natural products are available and require only sterilisation. Milk is a fluid well adapted for bacterial growth; potato is a good medium for some species, while solidified blood-serum is peculiarly fitted to provide nourishment for many of the parasites. More commonly, however, artificial media are employed. The foundation for most of those in general use is a meat broth, prepared by boiling good lean meat—*e.g.*, rump steak—in water. Boiling coagulates the albuminous material, and therefore it is desirable to fortify the broth in this respect by adding some soluble proteid such as commercial "peptone," and it is of advantage to add also a little common salt. Such a mixture contains all the elements requisite for the growth of most bacteria in a very favourable form. It has to be filtered till it is quite clear, and it must then be *neutralised*. The broth, as freshly prepared, is slightly acid. Now some bacteria can grow well in a slightly acid medium,

but most prefer a medium which is neutral or very slightly alkaline, so very diluted caustic potash or soda is added until the acidity, as tested by litmus paper (or some other indicator), is just overcome. The broth thus prepared is known as "bouillon" or "nutrient broth" or "peptone broth," and is very largely employed as a nutrient medium for bacteria.

By the addition of certain substances to this broth it can be made solid at ordinary temperatures, securing all the nutrient value of the broth with the advantages of a solid medium. The substances in common use for this purpose are "gelatin" and "agar-agar." The addition of 10 per cent. of the best gelatin yields a medium which should be perfectly transparent and which is solid below 77° F. Such *nutrient gelatin* is, on the whole, the most favourable solid culture medium at our command; that is to say, it is the medium on which the greatest number of bacterial species will grow to the best advantage. It has, however, certain drawbacks. It melts rather too easily; a gelatin tube held too long in the human hand may melt, and it is not rare for the temperature of the laboratory to rise during the hotter months of the year to more than 77° F. Gelatin tubes inadvertently left in the sun, or too near a Bunsen burner, may easily be melted. Thus the advantages of a solid medium may be lost and the cultures spoiled. Moreover, gelatin cannot be used for growing bacteria at the body temperature (except in the melted condition). On the other hand, the low temperature at which it becomes fluid is in some respects an advantage, being far below the thermal death-point even of non-sporing bacteria, so that cultures can be

melted and poured out as "plate cultures" (to be described below) without any risk of injury to the bacteria. Another disadvantage lies in the fact that some bacteria produce a ferment which liquefies the gelatin, spoiling it as a solid medium. In other respects this is an advantage, since the presence of the power of liquefaction, and its rate and mode, are useful aids in distinguishing between different sorts of bacteria.

The other substance commonly added to the broth medium to make it solid is "agar-agar," which is prepared from an Eastern seaweed, and is sometimes called "Japanese isinglass." It is added to the broth to the amount of 2 per cent., and this *nutrient agar-agar* is almost, though not quite, as favourable a soil for the growth of the majority of bacteria as the gelatin mixture just described. When once solid it requires a temperature little short of boiling to melt it again, though when melted it does not become solid till the temperature falls to 104° F. It can thus be used to grow bacteria at the body temperature, and there is no risk of its melting, however hot the weather. Further, it is not liquefied by the growth of any known bacteria. These two advantages are so great that this medium is perhaps more widely used than any other.

For the needs of certain special bacteria it is easy to add to any of the above standard media the particular substances they love. Thus, in order to grow the tubercle bacillus, the addition of some glycerin has been found advantageous, while to grow anaërobic bacilli grape-sugar is added. There is, indeed, no limit to the variations which may be introduced into the composi-

tion of culture media, but the above are the forms most generally useful.

In practice it is found convenient to put the media into glass vessels, each containing enough of the nutrient medium for a single "cultivation." As a rule, ordinary test-tubes are well adapted to this end, but where a large surface is required the melted medium is poured into shallow round glass dishes, provided with glass lids, and known as "Petri's capsules." Such "plate cultures" are prepared as they are wanted, and the glass lid preserves them from contamination by aërial germs for the short time they are required. But tube-culture media are prepared and stored for a long while, and after they are inoculated the cultures are often kept alive for many months. Their preservation from accidental contamination is therefore very important, and is assured by the simple device of plugging the mouth of the tube with cotton-wool (preferably the raw cotton-wool, not "absorbent wool"). A well-made cotton-wool plug allows access of air to the culture, but acts as a perfect filter against bacteria.

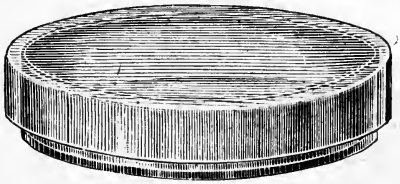

FIG. 11.—Double glass dish for plate cultivations (Petri dish). The larger dish fits as a lid over the smaller one. (Reproduced by the courtesy of Messrs. Baird and Tatlock.)

In the preparation of the culture-tubes absolute sterility is essential; unless they are perfectly sterile to start with, one cannot grow pure cultures in them. They are sterilised by heat. The test-tubes which are going to be used, having been first scrupulously cleansed inside and out, are put in a hot-air steriliser and heated

for an hour or so to a temperature of 400° or 500° F., which ensures the destruction of the most resistant spores. The cotton-wool to be used for the plugs is treated in the same way. The glass funnels, beakers and flasks which will be used in the final stages of the preparation of the culture medium are similarly sterilised. In the process of making the beef broth the ingredients are boiled several times over, but in the processes of filtering, neutralising, &c., there is necessarily some contamination by aërial germs, though this is as far as possible avoided. When the fluid is finally ready it has to be poured into the ready prepared test-tubes, and these must be then plugged with the sterile wool: here again some contamination must occur, but when once the cotton-wool is in place the culture-tubes are safe from further contamination, and now the final sterilisation takes place. This is effected by heating the tubes to boiling-point in a steamer similar in principle to the sort of vessel in which potatoes are cooked. The method employed is that of *discontinuous sterilisation* —viz., heating to boiling-point for three-quarters of an hour on each of three successive days. The principle involved is as follows: It is possible that some spores may be present which are so resistant as to escape destruction on the first boiling. This is not a mere supposition: it does at times occur. Now, when the fluid is cool again, such spores find themselves surrounded by all the conditions necessary for growth, and with no competition from other bacteria, for they are the sole survivors. After an hour or two they probably begin to germinate, and soon give rise to progeny. In time the resultant bacteria would in their turn go on to

form spores, but this does not occur at once, and if the boiling be repeated at the end of twenty-four hours (or less, in warm weather), the bacteria are caught in a relatively defenceless condition and succumb. To make quite sure, the boiling is usually repeated on the third day, and there are some fluids so difficult to sterilise—milk, for example—that it is even advisable to do it four times. The virtue of this method of discontinuous sterilisation is such that it can even be applied to media which will not stand actual boiling without deterioration—*e.g.*, blood-serum. The first heating aims only at killing the bacteria in the non-sporing stage: the spores will be dealt with after they have been germinated, on the second and third days. There is, therefore, no absolute necessity for raising the temperature in this method beyond that known to be fatal to non-sporing bacteria—*i.e.*, to 160° F. or thereabouts. The method is largely used for sterilising milk, and is called "Pasteurisation." When the culture-tubes have been thus three times heated they are stored away till wanted, and will keep good indefinitely, save that in time they will dry up by evaporation through the cotton-wool plug. Nutrient media may be preserved in bulk in large flasks, the necks of which are plugged with cotton-wool, and which have been steamed on three successive days. The medium can be melted and poured into tubes when wanted, but naturally the tubes prepared must again go through the sterilising process, and every time the stock flask is opened it must again be plugged with cotton-wool and resterilised. It is almost unnecessary to point out that the solid culture media, such as gelatin and agar-agar, have to be filtered

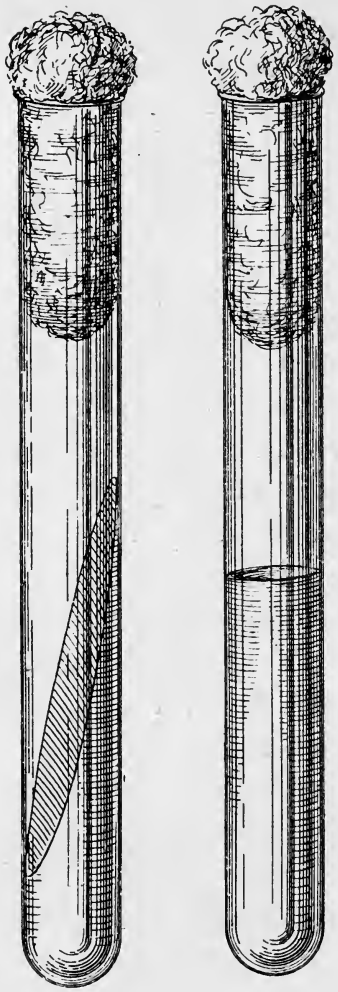


FIG. 12.—Sterilised culture tubes plugged with cotton wool. In (*a*) the medium has been allowed to solidify in a slanting position, to give a greater surface for "streak cultures." In (*b*) it has been solidified in the upright position, to give a greater depth for "stab cultures."

and poured into their tubes in the hot melted condition. The principles involved are the same as in the case of fluid media, only they are a little more troublesome to carry out. When such solid culture-tubes are finally sterilised they are allowed to solidify, either in the upright or in a slanting condition, according to requirements. When it is desired to grow the bacteria on the surface of the culture medium a much larger surface is obtained by sloping the tube before it sets. When it is wished to study the bacterial growth in the depth of the medium, a much greater depth is obtained by letting it set in the upright position. The cultures so made are known respectively as "surface cultures" or "streak cultures," and "stab cultures."

**Methods of Cultivation.**—The mode of employment of the prepared culture media must now be briefly described, but can only be learned by actual practice. Let us take the simplest

instance, in which we have a pure culture of a given organism in one tube and wish to transfer it to another in order to obtain a fresh culture. Let us further assume that it is a disease-producing organism liable to infect the incautious manipulator. The problem is a twofold one. It is necessary to accomplish the transference without the contamination of the culture from extraneous sources and without the infection of the operator. In order to secure these ends, a certain ritual is established, the due observance of which becomes, with a little practice, a matter of routine, but which has to be learned. No point in this ritual can safely be neglected, and the reasons for each step must be explained in a few words.

FIG. 13.—Glass capillary pipette, drawn out from a piece of glass tubing in the blowpipe flame. For use, the ends are broken off and the material to be inoculated is sucked up into one end of the pipette. The bulb in the middle prevents the risk of the passage of the material into the mouth.

A finely-pointed instrument is used in conveying the bacteria from one tube to another, since it is only requisite to transfer a very minute amount of the infecting material. A finely drawn out glass tube (a "capillary pipette") is sometimes used for fluid substances,

but more commonly a metal wire is used. Platinum wire is the best because of its indestructible nature; it can be heated to redness many times a day for years without being spoiled. The wires are mounted on glass handles, and two forms are in common use—viz., a *straight wire* where it is required to transfer only a very minute amount of the infecting material, and a *loop* where it is needful to transfer more. In using any form of wire it must be sterilised, by heating to redness, *both before and after use.* It is heated before use in order to protect the purity of the cultivation; even from a red heat sufficient cooling takes place in ten or fifteen seconds to render it safe to use; naturally the sterilised wire must touch nothing after heating till it is introduced into the culture-tube. It is heated again after use in order to protect the operator and prevent contamination of his surroundings. A wire which has been used for an inoculation must never be laid upon the bench or allowed to touch anything till it has been sterilised.

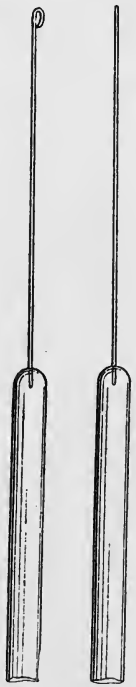

FIG. 14.—Platinum wires for inoculation, mounted in glass handles. The lower wire is looped at the end, in order to convey a larger amount of the material to be inoculated.

In order to make the inoculation the cotton-wool plugs have to be removed from both tubes. This is done with forceps—either ordinary or "dressing" forceps; it is not essential that the forceps should be

previously sterilised, though they should not be dirty; the plugs will have to be passed through the flame before being replaced, and this is a sufficient safeguard. The removal of the plugs entails exposure to the air, and hence a chance of contamination by aërial germs. The danger is not so great as might appear, and it is minimised by holding the tubes in a slanting position and by conducting the inoculation as rapidly as is consistent with due care. A little practice will enable any one to carry out the entire procedure in some twenty seconds. Now a sterile gelatin plate deliberately exposed to the air for two minutes usually develops only twenty or thirty colonies, though it has a diameter of four inches. It follows that a tube half an inch in diameter, held very obliquely and exposed for only twenty seconds, will only exceptionally be contaminated. The mathematical chances are more than twenty to one against contamination, and in actual practice it is found that in experienced hands very few tubes are thus accidentally infected.

The cotton-wool plugs, while removed from the tubes, may be held between the fingers or laid on a piece of wire gauze. In either case they must be held in the flame of a Bunsen burner or spirit lamp, and set on fire before they are replaced. Any smouldering patch may be extinguished after replacement by pressure with the forceps.

The actual inoculation is performed by touching the old culture with the sterile wire and thus removing a trace of the growth, which is then streaked over the surface of the sterile tube, or stabbed into the substance of the medium, as the case may be. The exact details

of the process must be learned by actual practice under the supervision of a teacher. The above remarks are only intended to indicate the principles upon which the operation is conducted. The principles are the same whether a solid or fluid medium is to be inoculated, and whether the culture-tube is inoculated from a previous pure culture-tube, from an isolated colony on a plate culture, or from any other source. The two objects which have always to be kept in mind are the avoidance of accidental and especially of aërial contamination, and the prevention of any risk of infection of the operator or of his surroundings.

**Plate cultures** are much used where a very large surface is required, or where it is necessary to study the individual colonies under a low power of the microscope. They are of value in separating out the different species from a mixture of bacteria. The "plates" in which they are carried out are shallow glass dishes of various diameters, provided with loosely-fitting glass lids of the same form: the larger dish fits over the smaller one. They are sterilised before use by heating in the dry-air steriliser. Ordinary prepared tubes of the culture medium (gelatin or agar-agar) are melted, and when the plate is cool the melted contents of the tube are poured into it and allowed to set as a thin layer all over the bottom. This is done with as little exposure to the air as possible, but the risk of contamination is greater than in inoculating ordinary culture-tubes. The medium may be inoculated in the melted condition before being poured out, or it may be inoculated on the surface only, after it has set. The object of plate cultures is, as a rule, to obtain isolated colonies from which pure cultures

may be started; if the colonies spring up too closely together this cannot easily be attained. A common device to separate a mixture of species in a culture is as follows: A little of the mixed growth of bacteria is put in a sterile broth tube and well shaken to separate the individual organisms. Three gelatin culture-tubes are melted in warm water. In the first gelatin tube are placed two or three platinum loopsful of the mixed broth emulsion, and it is gently shaken and mixed. From this two or three platinum loopsful are transferred to a second gelatin tube, and when this has been well mixed two or three loopsful are again transferred from it to the third gelatin tubes. The three gelatin tubes are now separately poured into three sterile plates and allowed to solidify. They represent three different degrees of dilution of the original mixture, and it is safe to prophesy that in one of the three (usually the second) the colonies will be sufficiently numerous for study, yet sufficiently far apart to allow of pure sub-cultures being made.

# V

## DISINFECTION BY HEAT

HEAT is the simplest and most generally applicable means at our disposal for the destruction of bacteria. For each particular sort there is, within fairly narrow limits, a temperature at which death occurs. The time of exposure to such temperature is, of course, an essential point. Higher temperatures kill more quickly than lower. It is customary to take ten minutes as a convenient time limit; adopting this, it is not difficult to determine very closely the exact temperature at which any given species perishes, and this temperature is called the *upper thermal death-point*. There is no "lower thermal death-point" for bacteria, or at least it has not yet been attained; they can withstand exposure to more than 100° below zero and yet revive on thawing.

The most striking fact concerning the destruction of bacteria by heat is the great difference between the thermal death-point of spores and of the non-sporing forms. As a general law, it may be stated that, whereas non-sporing bacteria (that is, bacteria which never form spores, and the growing or vegetative forms of those which do) are certainly killed by a genuine exposure to 170° F. for ten minutes, true spores require exposure to 212° F. for a time varying, according to

the species, from a minute or two up to half an hour for their certain destruction. A second fundamental fact is that both non-sporing bacteria and spores are much more resistant to heat in the dried condition than when moist. Unless the contrary is expressly stated, the upper thermal death-point must be understood to imply moist heat. The statements must now be illustrated by actual facts.

**Thermal Death-points of non-sporing Bacteria.**—The following figures are selected from the results of Sternberg's well-known and accurate experiments :

| | | | |
|---|---|---|---|
| Cholera vibrio | killed at | 52° C. (125°.6 F.) | in 4 minutes. |
| Pneumococcus | „ | 52° C. (125°.6 F.) | 10 „ |
| Streptococcus pyogenes | „ | 54° C. (129°.2 F.) | „ |
| Typhoid bacillus | „ | 56° C. (132°.8 F.) | „ |
| Staphylococcus pyogenes aureus | „ | 58° C. (136°.4 F.) | „ |
| Sarcina lutea | „ | 64° C. (147°.2 F.) | „ |

These figures represent the range of temperature within which non-sporing bacteria are killed. (For the methods of testing thermal death-points see experiments on p. 186, *et seq.*) Sarcina lutea, a harmless saprophyte, has an exceptionally high resistance. Amongst disease-producing bacteria which form no spores, Staphylococcus pyogenes aureus has the greatest resistance to heat, and the writer has met with strains of this coccus which have a somewhat higher resistance than Sternberg's figures illustrate. As a matter of fact, slight individual variations in resistance are common enough in different strains of the same species.

There exists, however, a group of micro-organisms, typified by the genus Streptothrix, and commonly

classified with the bacteria (see p. 23), which form spores of a kind, though not by any means of the resistance of the bacterial spores. In this group the thermal death-point is a little higher than in the true bacteria. Some forms of the ray-fungus require nearly half an hour's exposure to a temperature of 167° F. for their destruction. The tubercle bacillus is on many grounds believed to belong to the Streptothrix group rather than o the true bacteria, and it resembles this group in its thermal death-point. It is inferior in its resistance to true bacterial spores, but it resists heat better than ordinary non-sporing bacteria. Its resistance may in part depend upon the non-conducting properties of the fatty matter which enters so largely into its composition.

**Thermal Death-point of true bacterial Spores.**—Of all known forms of living matter these possess the most marvellous resistance against heat. They probably owe this property to the impenetrability of their dense envelope. They can resist the temperature of boiling water for periods varying, in different species, from one to two minutes up to half an hour, or in rare cases even longer. It is fortunate that the species of spore-bearing bacilli which are known to produce disease in men (anthrax, tetanus, &c.), are killed by boiling in five or ten minutes at most. At temperatures higher than that of boiling water (*e.g.*, steam under pressure) they are still more quickly killed.

Dry heat, on the other hand, is far less effective in killing bacteria. Even non-sporing forms have been found to resist a temperature of over 212° F. for an hour when they were in the dried state, while Koch

and Wolffhügel found that, to make certain of destroying spores in the absence of moisture, an exposure to a temperature of 284° F. for no less than three hours was necessary.

From the above facts it is plain that spores require far more energetic measures for their destruction than non-sporing bacteria. Now in practical disinfection it is not safe to assume that the bacteria present are non-sporers. In certain special cases, as where a book has been used by a diphtheria patient, or a sheet soiled by typhoid excreta, it may be permissible to act on such an assumption, because we know that the diphtheria bacillus and the typhoid bacillus are not spore-formers. But in actual practice heat disinfection is directed against spores, in order to be on the safe side, for in many diseases the nature of the microbe is unknown.

* * * * *

The problem of heat disinfection is thus to secure, in reasonable time, the access to every part of the material to be disinfected, of a temperature adequate to destroy the spores of all pathogenic bacteria. It must be borne in mind that many substances requiring disinfection are bad conductors of heat. Fatty materials conduct heat very badly; it is far more difficult to sterilise milk than water, and cream than milk, because the bacteria are apt to be lodged in the middle of fat globules. All solid masses are more difficult to disinfect than fluids, because the heat penetrates slowly into their interior.

The methods employed in heat disinfection are (1) dry heat; (2) boiling; (3) steaming.

**Disinfection by Dry Heat.**—In the laboratory dry heat is largely used. Instruments of little value

are commonly heated for a short time in the flame of a Bunsen burner or spirit lamp. Small hot-air sterilisers, consisting of copper or sheet-iron chambers, heated from below by gas-burners, are of the greatest utility

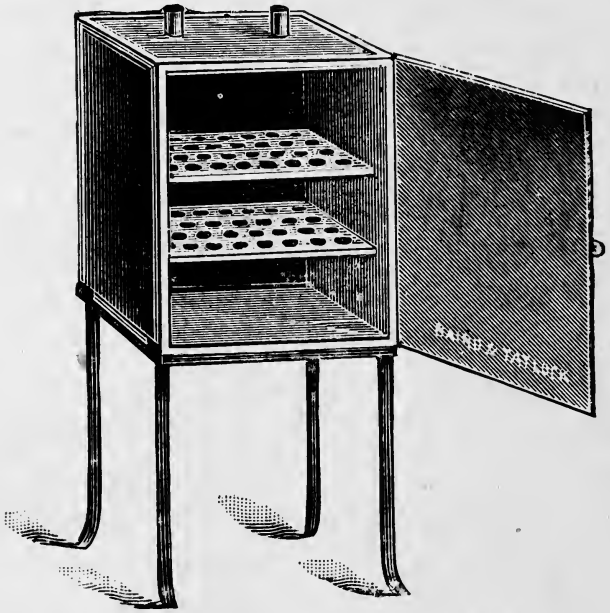

FIG. 15.—A simple form of hot air steriliser. It is made of sheet iron or copper, with double walls, and is heated from below by a Bunsen gas burner. (Reproduced by the courtesy of Messrs. Baird and Tatlock.)

for the sterilising of glass apparatus, metal instruments and loosely packed cotton-wool. A temperature of 400° F. is easily attained in them, and is amply sufficient to destroy the most resistant spores in a very short time. Surgical instruments are sometimes treated in the same way. Cases containing scalpels, constructed of metal throughout, can be heated over a spirit lamp. But for instruments of any value the method is not good, as the temper of the steel is apt to be destroyed by frequent heating, and it is difficult to keep them sharp.

On a larger scale, dry heat is still sometimes employed for the disinfection of clothing and bedding. Before the introduction of steam disinfection it was largely used. The apparatus consists essentially of a large chamber or oven, the desired temperature being attained by a furnace beneath it, or, better, by rings of gas-burners. The practical objections which have led to the substitution of steam disinfection for dry heat are two in number. In the first place, the high temperatures required for complete disinfection (280°–290° F.) are found to damage and discolour the fabrics submitted to it: most materials will stand 250° F. without injury, but above this, scorching, shrinking, and a diminution in the strength of the fabric are apt to take place. In the second place, and this is a more important objection, dry heat penetrates with extreme slowness into the interior of bulky objects, such as bundles of clothing, pillows and mattresses. We select, in fact, for our clothing and bedding, materials which are bad conductors of heat, in order to keep ourselves warm, and this fact renders them more or less impenetrable to heat from without. Numerous experiments have shown that a thermometer placed in the centre of a pillow exposed to a dry temperature of 250° to 300° F. for an hour or more may register more than 100° lower than the air in the oven, though the outside of the pillow may be scorched. It is probably impossible to sterilise the interior of a mattress by dry heat without seriously damaging its outer covering.

**Disinfection by Boiling.**—It has been stated that non-sporing bacteria are destroyed by moist heat at a temperature far below that of boiling water, while

the spores of all known pathogenic bacteria are killed by five or ten minutes boiling. These statements require some qualification. The temperature at which water boils is dependent upon the pressure under which it is heated: the lower the atmospheric pressure, the lower is the temperature of ebullition. It is only at sea-level, or with a barometric pressure of thirty inches of mercury, that water boils exactly at 212° F. In ascending a mountain the barometric pressure becomes less and less, and water boils at a lower and lower temperature. On the top of Mont Blanc water would boil at 183° F. Conversely, the boiling-point of water can be raised by raising the pressure under which it boils. By causing it to boil in a closed vessel the pressure may easily be raised to double the normal or more: at a pressure of two atmospheres (30 lb. to the square inch) water boils at 249° F. The vessel must be a strong one or it may burst: it is necessary to provide a safety-valve. But the resistance of bacteria to heat is not affected by atmospheric pressure, so that up in a balloon it might be difficult to kill spores at all by boiling except under pressure. Darwin states that in crossing the Chilian Cordilleras he found it impossible to cook potatoes, by boiling, at an elevation of 11,000 feet. Statements, therefore, as to the destruction of bacteria by boiling must be understood to apply only to boiling at moderate altitudes above sea-level.

With this reservation, boiling is an excellent and safe method of sterilising such objects as are not injured by the process. Surgical instruments constructed entirely of metal, the filtering candles of modern high-pressure filters, glass objects, &c., can all be disinfected in this

way. Many woven fabrics can be so treated, though for these steaming is to be preferred. There are other materials which cannot be so treated: sea-sponges, for example, are spoiled by boiling, losing their elasticity of texture, while objects into the construction of which wood and similar material enters are clearly unfitted for the process. There is a simple means by which the practical efficacy of boiling may be increased. The solutions of various salts boil at a somewhat higher temperature than pure water. The addition of a harmless salt, such as carbonate of soda, to water slightly raises the boiling-point, and hence increases the disinfectant action. At the same time, metals are less injured than by boiling in pure water, while it is probable that the soda assists the penetration of the coats of the bacterial spores. Where it is desired to sterilise a test-tube in a hurry, as often happens when pus or some other product needs to be preserved for bacteriological examination, in the course of a surgical operation, it may be simply done by half filling a clean test-tube with water, loosely plugging the mouth with cotton-wool, and then boiling it for a few minutes over a spirit lamp—the steam escaping through the cotton-wool plug. The water can then be poured away, the plug quickly replaced, and the tube allowed to cool. (For experiments on the destruction of spores by boiling, see p. 187.)

**Disinfection by Steam.**—Of all methods of disinfection by heat this is the most efficacious and generally applicable. Moist heat kills bacteria at a considerably lower temperature than dry heat, while steam, under suitable conditions, has a power of

penetration into the interior of objects to be disinfected which vastly exceeds that possessed by dry air. The reason for this power of penetration must shortly be explained.

Let us assume that water is boiling at ordinary atmospheric pressure—*i.e.*, at a temperature of 212° F. If a vessel containing a pound of water at freezing-point be heated over a fire, the course of events is as follows: The heat from the fire is communicated to the water, and gradually raises its temperature till it reaches the boiling-point. So far the heat from the fire has been spent in raising the temperature of the water from freezing-point to 212° F., but when once boiling begins no further rise in temperature occurs. However hot the fire, the temperature of the water will never rise above 212° F. if the steam can freely escape and the atmospheric pressure is normal. Thenceforward the heat of the fire is all expended in converting the water into steam. Yet the steam is no hotter than the water—both are at 212° F. The energy expended in doing this has disappeared as heat—that is, has caused no further rise in temperature, but it is not lost, for energy is indestructible. It has merely taken another form and is said to have become "latent," and when the steam condenses again into water, this latent heat is all given out again as real effective heat. The latent heat of steam is very great. Physicists are able to calculate, with great exactness, the amount of heat required to raise a pound of water from the freezing-point to the boiling-point: they can calculate equally precisely the amount of heat used up, or becoming "latent," in converting a pound of water at

212° F. into steam at 212° F. They have proved that it takes more than five times as much heat to convert the pound of water into a pound of steam, as it does to raise the pound of water from freezing-point to boiling-point. So that, when the pound of steam again condenses into water, it gives out, as effective heat, more than five times as much as would raise this amount of water from 32° F. to 212° F. In the light of these facts the enormous penetrating power of steam heat can easily be understood. Suppose steam, at ordinary atmospheric pressure, to be brought into contact with a pillow or a mattress. It immediately undergoes condensation on touching the outer surface of the colder object, and in so doing parts with its latent heat, which is more than sufficient to raise this outer layer to 212° F. In condensing, steam shrinks immensely in volume: a cubic foot of steam condenses into about a cubic inch of water, so that a partial vacuum is formed by condensation, into which fresh steam rushes, repeating the process in the next layer of the pillow or mattress, and thus, layer by layer, the entire object is raised to a temperature of 212° F., whether it is a good conductor of heat or not, and this in a few minutes. Hot air, on the contrary, can penetrate only in virtue of the power of the material to conduct heat, which, as has already been stated, is, in most objects requiring disinfection, comparatively low. The virtue of steam depends upon the fact that it parts with its great latent heat when it condenses.

Very simple steam sterilisers are in use in laboratories for the preparation of culture media. They are made of copper or sheet-iron and have a loosely-fitting lid.

Water is poured into the bottom to a depth of about two inches, and is boiled by heat applied underneath the vessel from gas-jets. A layer of wire-netting separates the water from the objects to be sterilised, which are thus immersed in steam from water boiling under ordinary atmospheric pressure, and quickly attain its temperature—approximately 212° F. The waste steam escapes through a hole in the lid of the vessel. The principle is simply that used in every kitchen for steaming vegetables. An even more efficient apparatus is the *autoclave*. This is a much more strongly-made metal cylinder, provided with a hermetically-fitting cover which can be secured in position by a series of screw clamps. As in the simple steam steriliser, a layer of water some two inches in depth is boiled by a ring of gas-jets beneath the vessel, but there is this difference, that the steam cannot escape, and hence the pressure inside the cylinder rises. As it rises, the temperature at which the water boils, and hence that of the steam, rises too. A pressure-gauge indicates the exact pressure, and hence the exact temperature, inside the apparatus. A safety-valve is provided, and a tap by

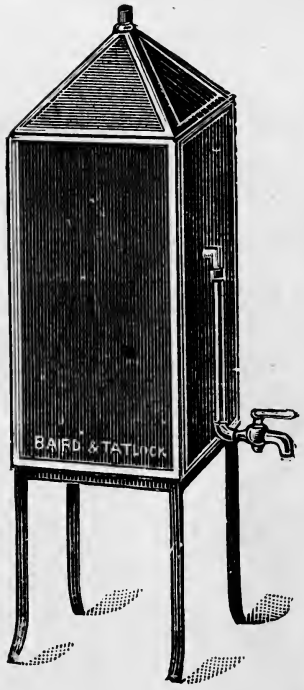


FIG. 16.—A simple steam steriliser made of sheet copper covered with felt, heated from below by Bunsen gas burners, and fitted with a water tap and gauge, so that the amount of water in its lower part can be readily seen. (Reproduced by the courtesy of Messrs. Baird and Tatlock.)

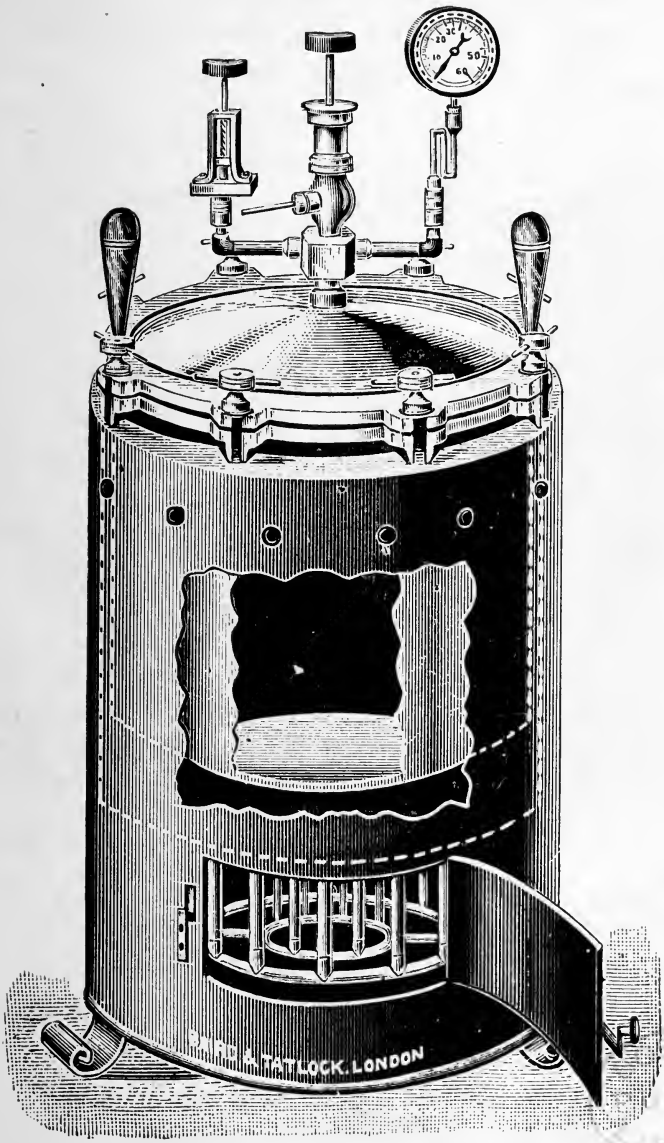


FIG. 17.—Autoclave, for sterilising by steam under pressure. It is strongly constructed of iron and fitted with a pressure-gauge and safety-valve. The arrangements are shown by which the lid is clamped into position, and the inner vessel is heated from below by a ring of Bunsen gas burners. (Reproduced by the courtesy of Messrs. Baird and Tatlock.)

which the steam can be allowed to escape. The apparatus can be worked very conveniently up to a pressure of 30 lb. on the square inch, yielding a temperature of 249° F. There is little advantage in

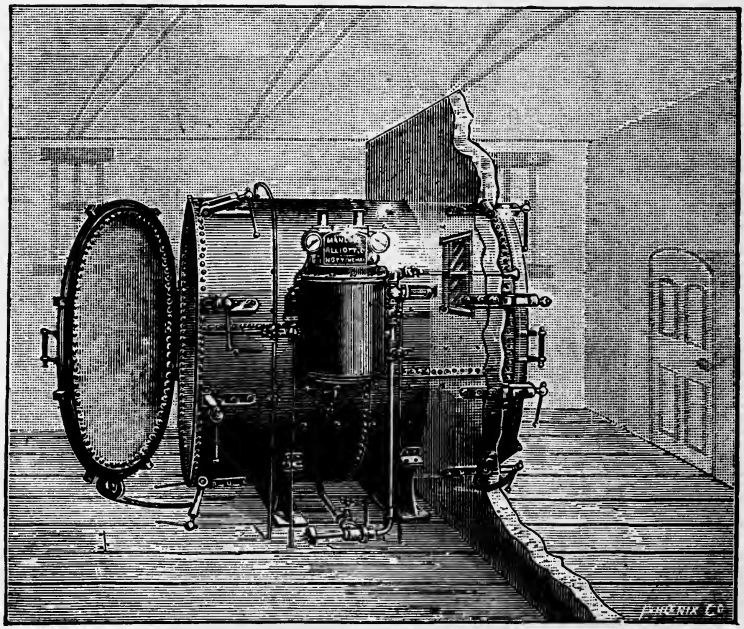

FIG. 18.—One of the forms of the "Washington Lyon" high pressure steam disinfecting apparatus. The large oval cylinder is double jacketed, and strongly constructed of iron. It is seen passing through the wall between two adjacent rooms. This type of instrument, fitted with a vacuum apparatus, is of a high degree of efficiency. (Reproduced by the courtesy of Messrs. Manlove, Alliott and Co.)

working it at any higher pressure than this. The most resistant spores are quickly killed, and the penetrating power of the steam ensures the complete disinfection of even bulky objects.

Apparatus of this kind is, however, only suited for disinfection on a small scale. Where it is required to disinfect bedding and clothes in bulk, correspondingly

large apparatus is necessary, and steam disinfectors have now been brought to a pitch of great efficiency. Many different forms are in the market, varying in cost up to several hundred pounds. It is unnecessary to describe here any one apparatus in detail. The general principle is much the same in all, and is as follows: The steam is generated in a boiler, at a pressure in accordance with the temperature desired; a pressure of two atmospheres is ample for most purposes. From the boiler it passes to the disinfecting chamber, which is sometimes constructed as a double cylinder—an inner one containing the goods to be sterilised, surrounded by an outer steam-jacket. The two ends of the cylinder should open into two different rooms, the apparatus passing through the wall between them; thus the sterilised articles are taken out into an uncontaminated room. The exact method of working differs in different forms of apparatus. In some the goods are warmed up by allowing steam to circulate in the outer jacket before the steam is turned on into the inner chamber. In some there is an arrangement by which the air is exhausted from the inner chamber before the steam rushes in, thus facilitating penetration, while by the same arrangement the steam can itself be sucked out when disinfection is complete, thus facilitating the drying of the articles. But in all the essential principle is the same—the rapid raising of the temperature of the goods to be disinfected by the *condensation* of the steam upon them, whereby it parts with its latent heat.

It is to be noted that the steam employed in all these forms of apparatus is what is called "saturated steam," that is, steam at or near its condensation-point. The

actual temperature of the steam will vary with the pressure; for every pressure there will be a temperature at which the water naturally boils and condenses again. But it is possible artificially to raise the temperature of steam beyond that natural to it at the pressure under which the water has boiled. This has to be done by some extra supply of heat from another source: steam can be heated up to any desired temperature just as air or any other gas can be heated. Such steam, heated above its natural condensation-point, is called "superheated steam." Under a pressure of two atmospheres the natural temperature of the steam would be 249° F.; and it would still be saturated steam, ready to condense the moment the temperature fell below 249° F. But under a pressure of one atmosphere the natural temperature of the steam would be 212° F.; if now by artificial means, and without altering the pressure, such steam were raised to a temperature of 249° F., it would be superheated steam. The physical difference between the two sorts of steam would be that the superheated steam would not condense till its temperature fell from 249° to 212° F. It would have lost its faculty for ready condensation and ready giving up of its latent heat. Therefore saturated steam is greatly preferred for disinfecting purposes. Superheated steam has not much greater power of penetration than air at the same temperature.

The efficiency of disinfection by a properly constructed steam apparatus is very great. If the steam penetrates, sterilisation is absolute; the most resistant spores are certainly destroyed. And the steam does penetrate when the apparatus is properly worked.

This may be tested by placing registering thermometers in the centre of mattresses, bundles of clothing, or big books. Numerous experiments prove that a temperature of 230° to 250° F. is easily attainable in the centre of such objects, and bacterial spores placed in such situations are always found to have been killed. (For a practical test illustrating this, see p. 189.) For the absolute disinfection of ordinary objects we are therefore justified in placing complete reliance on steam disinfection. Nevertheless there are limits to the penetrating power of steam. One would not expect a large block of wood to be penetrated to its centre, and objects are sometimes met with as hard to disinfect as this. Horsehair is sometimes imported in great bales which have been compressed by hydraulic power into solid blocks which steam cannot penetrate. The writer has had occasion to test such a bale, infected with anthrax, after it had been exposed to the action of a Washington-Lyon steam disinfector. Even non-sporing bacteria had survived in its centre.

They are many objects for which steam disinfection is unsuitable. It ruins things made of leather or felt. Paper itself is scarcely injured, but the covers of books are spoiled. The process is not well adapted for metals and wooden articles. Its great field is in the disinfection of bedding, clothing, carpets, curtains and textile fabrics generally. None of these should suffer any injury, and they are precisely the things which are most difficult of penetration by other disinfecting agencies. Warning must, however, be given as to the fixation of stains by steam, as by other methods of disinfection by heat. It commonly happens that pillow-cases, sheets and blankets

are soiled by blood, fæcal material, or urine. Such stains are indelibly fixed by steam disinfection, and soiled articles should therefore be soaked in water (to which a disinfectant, such as 1 part in 100 of carbolic acid, or 1 part in 200 of izal, has been added) for twenty-four hours before they are steamed. The fixation of stains can thus be in large part, though not wholly, avoided.

A rough-and-ready way of disinfecting pails or other metal vessels which have been used for the conveyance of infected material is to invert them, after they have been emptied of their contents, over a jet of steam from a boiler.

# VI

## DISINFECTION BY CHEMICALS

A GREAT many chemical substances have an injurious effect upon living protoplasm. In other words, they act as poisons to living cells, in various degrees. Some kill outright, some paralyse without actually killing, while the feebler poisons merely interfere with the performance of the higher functions of life. As a rule, the chemical substance which is poisonous to one kind of protoplasm is poisonous to all, though not always to the same degree. The discovery of a substance which would act as a poison upon the bacterial cell without any power of injuring the cells of the human body would solve many difficult problems in disinfection. Unfortunately, the substances which kill bacteria also tend to kill man, though, naturally, man requires a proportionately larger dose. This danger in the employment of disinfectants must always be borne in mind, as fatal results have sometimes followed their incautious use.

The effects of chemical poisons upon bacteria may be divided into three different grades, distinguished by different terms:

(*a*) A very feeble poison, or an extremely weak solution of a stronger poison, may not altogether prevent the growth and multiplication of bacteria, but may yet

interfere with their highest vital activities. If the anthrax bacillus be grown in broth to which a very minute trace of carbolic acid has been added (1 part in 1000) multiplication is not prevented, but the bacillus loses its power of forming spores, and in time it loses also its power of producing poisonous substances, so that it ceases to be "virulent" when injected into an animal. Its virulence, or power of giving rise to disease, is said to be "attenuated." The power of causing disease is one of the highest and most delicate properties of pathogenic bacteria, and the most easily interfered with by injurious agencies. The mildest degree, therefore, in which a chemical poison can exert its action upon disease-producing bacteria is that of mere *attenuation* or mitigation of virulence.

(*b*) A rather stronger poison, or a less diluted solution of a very powerful one, may, while still unable actually to kill, entirely check the growth and vital activity of bacteria. Moderate doses of chloroform will put bacteria to sleep and keep them quiet, just as in the case of a human being. Carbolic acid, in the strength of 1 part in 500, is sufficient to prevent the growth of nearly all bacteria, while the more powerful perchloride of mercury can check growth even in so dilute a solution as 1 part in 50,000. This degree of chemical influence upon bacteria is called the *restraining* or *inhibiting* effect, and the term *antiseptic* should properly be limited to this degree of action, for it means that the substance employed antagonises "septic" or putrefactive change, without necessarily killing the bacteria. (Practical exercises illustrating restraining action will be found on pp. 190 and 191.)

(*c*) The more powerful poisons in sufficiently strong solution actually kill bacteria, and this constitutes truly *disinfectant* or *germicidal* effect. It will presently be seen that spores take a good deal more killing than the growing forms of bacteria: a disinfectant that will kill spores is sometimes spoken of as exercising a "complete" disinfectant action, while one that will kill the growing forms, but not the spores, is spoken of as an "incomplete" disinfectant.

It is important to bear in mind these three degrees of chemical action upon bacteria, and we are specially concerned here with the last two. Not only do different chemicals act in different degrees, but the same chemical may act in different degrees according to its concentration. Carbolic acid, in a strength of 1 in 20, will kill anthrax spores in a certain number of days, and non-sporing anthrax bacilli in a minute or two; in a strength of 1 in 500 it will prevent their growth; and in a strength of 1 in 1000 it will merely attenuate their virulence. So that carbolic acid is a complete or incomplete disinfectant, an antiseptic or a mere attenuating agent, according to the dilution in which it acts.

Another point which must never be forgotten is that a certain *time* is requisite for the destruction of bacteria by chemical agencies, as well as a certain *strength of solution*. Some people seem to imagine that, when a substance has once been proved to be a disinfectant, it does not matter how it is used. It thus is in danger of becoming a mere fetish. But carbolic acid and perchloride of mercury are not disinfectants in virtue of the magic of their names. They disinfect only when

present in a given effective strength of solution for a given period of time; if these necessary conditions are not complied with, they may be worthless.

Yet another consideration must be taken into account, namely the nature of the medium in which the disinfectant is to act. It is of primary importance that the poison should be in a soluble form, otherwise it cannot attack the bacterial protoplasm. If any substance is present in the medium to be disinfected which can chemically interact with the poison and precipitate it in an insoluble form, the disinfectant action is hindered or abolished. One of the most widely used disinfectants —perchloride of mercury—is open to an objection of this kind; albuminous materials combine with it to form a comparatively inert and insoluble substance. Pus, discharges from wounds, blood and animal juices in general, contain much albumin, and this neutralises much of the disinfectant virtue of the perchloride of mercury. If, therefore, one were to add to a given volume of pus, an equal volume of 1 in 1000 perchloride of mercury, the *effective* disinfectant value of the mixture would not be 1 in 2000 of the mercuric salt, but something very much less. Experiments with perchloride of mercury prove that its poisonous action upon bacteria gets less and less the larger the amount of albuminous material present in the medium in which the bacteria are suspended. Even the nutrient broth used as a culture medium contains enough albuminous matter to interfere appreciably in this manner with the disinfectant action of perchloride of mercury. A broth culture of Staphylococcus aureus is not, as a rule, completely disinfected by it in less than half an hour

(the perchloride being present in a strength of 1 part per 1000 of the total mixture). Most of the cocci in the culture are, indeed, killed in much less time, but some few hardy ones survive, and strains of this Staphylococcus may be met with which resist even longer than half an hour. But, suspended in distilled water, they are killed in a much shorter time (see p. 195).

Even apart from the formation of an insoluble precipitate, or an inert substance, a very marked influence upon disinfectant action may be exerted by the medium used in dissolving the chemical substance employed, or even by the simultaneous presence of some other, apparently indifferent, chemical body. The question is one of such practical importance that it will be necessary to give a short explanation of the possible reasons for the facts to be stated before mentioning the facts themselves.

**Ionisation.**—The old opinion about solution was that when a substance dissolved in water, its ultimate particles (which chemists call "molecules") separated from one another and swam about in the water without themselves undergoing any essential change. There are some inert and stable substances of which this is doubtless true—it seems to be true, for example, in the case of sugar. But there are also a large number of bodies of which it is now known not to be true or only partly true. These are the substances called "electrolytes"—that is to say, substances of which the solutions in water can conduct electricity. All the important chemical disinfectants belong to this group. When an electrolyte is dissolved in water a certain number of its ultimate particles (a number varying with the nature

of the substance, with the medium in which it dissolves, and with the presence of other chemical bodies) not merely swim about in the fluid, but are themselves separated into two parts, called *ions*, one of which bears a positive and the other a negative charge of electricity. This process is called *ionisation*, and it lies at the very root of chemical disinfection. It will be best understood by taking a simple example, such as perchloride of mercury. The ultimate particles, or molecules, of this salt consist of one atom of mercury combined with two atoms of chlorine. While all these atoms are firmly combined together to form the salt, the molecule is not only in a state of electrical equilibrium, but also in a state of chemical equilibrium; it cannot attack bacterial protoplasm or any other substance, and cannot therefore act as a disinfectant. But when it is dissolved in water, a certain proportion of the molecules are broken up into free mercury ions (bearing a positive electric charge) and free chlorine ions (bearing a negative charge). So that there are, swimming in the water, some ions of mercury and some of chlorine, together with many unaltered particles of perchloride of mercury. It is now in a condition to set up active chemical changes, and it is chiefly the free mercury ions, released

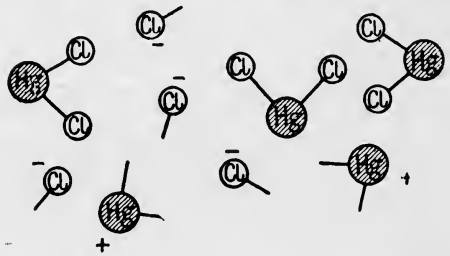


FIG. 19.—Imaginary diagram of a solution of perchloride of mercury in water. The atoms of mercury are represented by the larger shaded circles marked "Hg": the chlorine atoms by the smaller unshaded circles marked "Cl." Some of the mercury atoms are depicted joined on to two chlorine atoms to form the salt. Some are depicted as dissociated "ions" swimming about in the free state. The signs + and − attached to these indicate positive and negative electrical charges.

from their marriage bonds with the chlorine and eager to contract new alliances, which are responsible for the attack on the bacterial protoplasm, and thus for the disinfectant virtue of the solution. It follows that this disinfectant action depends entirely on the degree to which ionisation takes place, and this is found to vary widely according to circumstances. It can be shown by calculation that, in the presence of other chlorides, such as common salt, the number of free mercury ions should be diminished, and as a matter of fact it has been proved that the disinfectant virtue of perchloride of mercury steadily diminishes as more and more common salt is added to its solution. When perchloride of mercury is dissolved in pure alcohol, very little ionisation takes place. The spirit solutions of perchloride and biniodide of mercury, which are so much in vogue, owe what virtue they possess almost entirely to the spirit and not to the mercuric salts. Tested upon spores, which spirit alone cannot destroy, they are found to be valueless, but they kill non-sporing bacteria rapidly, just as strong spirit does. (See experiment on p. 197.) This is equally true of carbolic acid. Dissolved in water it is a very useful disinfectant, but dissolved in spirit it has no more value than spirit alone, and dissolved in oil it has no value whatever. It will thus be apparent that the vehicle in which a "disinfectant" is to act is of very cardinal importance, and it is a good general rule that it is safest to rely on watery solutions, and to avoid alcohol, oil or glycerin as solvents.

The difference between bacterial spores and vegetative bacteria is no less marked in their resistance to chemical agents than has been seen to be the case in their

resistance to heat, and for a similar reason. The living protoplasm inside the spore is probably as sensitive as that of the ordinary bacterium; it is the thickness and impermeability of the spore capsule which hinders the access of the poison. Even the most active germicides in ordinary use—watery solutions of the perchloride and biniodide of mercury—require an hour or two, in the customary strength of one part per thousand, to kill anthrax spores. The non-sporing bacteria are, with rare exceptions, killed by such solutions in a few minutes. It has already been mentioned that 1 in 20 carbolic acid takes several days to kill anthrax spores. These figures refer to disinfection at the ordinary temperature of the air. The high resistance of spores to disinfectant agencies is a serious bar to complete chemical disinfection, and in actual practice steam disinfection is much to be preferred where it is necessary to destroy spores. Much, however, of the routine disinfection required in practice is directed against bacteria which form no spores. Surgical disinfection, for example, in the preparation for, and conduct of an operation, is almost entirely aimed at streptococci and staphylococci; the routine measures taken in the preparation of the skin and hands are not such as to bring about the destruction of spores, yet in practice they give very satisfactory results, and should any serious infection chance to occur, it is hardly ever by a sporing organism. It is probable that any attempt chemically to destroy spores in the skin would seriously injure the skin itself. Again, in medical practice, many of the commonest infectious diseases are known to be due to bacteria which have no power of forming spores.

This is the case in cholera, typhoid, diphtheria, and probably in scarlet fever, measles, and the majority of zymotics. In small-pox it is probably not the case, so that here more severe disinfectant measures are advisable; but with this exception, and that of certain other fevers whose bacterial cause is as yet unknown, there is an immense field in medicine for chemical disinfection.

The list of chemicals which may be employed as disinfectants is a long one, and new substances are constantly being introduced, usually with a flourish of trumpets. But the number of substances proved by experience to be of real practical utility in chemical disinfection is comparatively restricted. The criteria by which a disinfectant is to be judged for practical purposes are shortly as follows:

(1) It must be truly germicidal within a reasonable time limit. For sterilising the hands and skin we require a disinfectant which will kill non-sporing bacteria in at most five minutes. For soaking infected linen we want one which will kill in a few hours. The actual germicidal power must be tested by accurate laboratory experiment carried out under conditions similar to those which will be met with in actual practice.

(2) It must not possess chemical properties which unfit it for ordinary use. The strong mineral acids and alkalies, though powerful disinfectants, are unfitted for everyday use by the corrosive action which they exert upon metals and other substances even in moderately weak dilution. It is a serious objection to perchloride of mercury that it damages metals, and that it forms

a relatively inert compound with the albuminous substances so commonly present in material requiring disinfection.

(3) It must be soluble in water, or capable of giving rise to soluble products in contact with the material to be disinfected.

(4) It must not produce too injurious an effect upon the human tissues with which it comes in contact. It is too much to ask that it should be non-poisonous to man, but the less poisonous it is the greater its sphere of utility as a gargle or internal disinfectant, and the less the dangers that will follow its external application to wounds. Carbolic acid in efficient germicidal strength produces an unpleasant effect even upon the healthy skin if the immersion is too prolonged or too frequently repeated, and the same is true of perchloride of mercury.

(5) It must not be too costly in proportion to its germicidal value. It is evident that a substance which will be efficient in a strength of 1 in 1000 can afford to be fifty times dearer than one which will be efficient only in a strength of 1 in 20.

* * * * *

It is proposed here to give authentic statements concerning a few of those substances which experiment has shown to be of real value as disinfectants, and to state the drawbacks which attend their use. It is only by an intelligent knowledge of the properties of these substances that they can be profitably employed.

**The Mineral Acids.**—Sulphuric, nitric and hydrochloric acids are extremely powerful disinfectants, but their range of application is very limited on account

of their corrosive action. Solutions of 8 or 10 per cent. are required to destroy spores in reasonable time (a few hours), but non-sporing organisms are killed by weak solutions, such as 1 in 200. Of the three acids named, nitric is the most powerful disinfectant and sulphuric the least powerful. Where it is requisite to carry out an energetic disinfection in a short time, and where the process can be conducted in glass or earthenware vessels, the addition of 10 or 20 per cent. of a strong mineral acid is an efficient means, but the fluid should not subsequently be poured down a sink or water-closet without very free dilution lest the metal pipes be damaged. One of the simplest means of sterilising a test-tube or bottle is to rinse it with a pure mineral acid and then get rid of the acid by repeated rinsings with boiled water.

**Metallic Salts.**—Of these, the only ones in general use are those of mercury, and especially the perchloride and biniodide. The salts of mercury are not of equal germicidal value. The careful experiments of Krönig and Paul show that the perchloride is the most efficient, the iodide less so, and the cyanide much less so.* Not

* In the majority of text-books it is asserted that biniodide of mercury is a more powerful disinfectant than perchloride. The writer's own observations, and a number of experiments carried out at his request by Mr. G. E. Barry, have convinced him that Krönig and Paul are right, and that the biniodide is considerably inferior to the perchloride in disinfectant power. It is alleged that the biniodide is not precipitated by albuminous material to the same extent as the perchloride. It is true that the bulky precipitate, which occurs when the biniodide is added to nutrient broth, soon re-dissolves, while the perchloride causes permanent turbidity. But experiment proves that even in broth the perchloride is the more active germicide. The re-solution of the biniodide precipitate does not imply a full recovery of disinfectant power.

only is the biniodide intrinsically less powerful than the perchloride, but it is subject to this further disadvantage, that it is scarcely at all soluble in water: in order to get it into solution some iodide of potassium has to be added, and this interferes to some extent with its ionisation, and hence with its disinfectant action, just as common salt interferes with the action of perchloride of mercury. The perchloride, however, will dissolve in pure water, so that this drawback is avoided. In spite of certain disadvantages, about to be mentioned, the intense germicidal activity of the mercuric salts places them in the front rank of practical disinfectants.

**Perchloride of Mercury** (Corrosive Sublimate) kills anthrax spores in a few hours (one to three), in watery solution, when present in an effective strength of 1 part in 1000. In this strength most non-sporing bacteria are killed in less than a minute (see p. 194), (but there is one very important organism, the Staphylococcus pyogenes, which has a peculiar resistance against this disinfectant and may survive exposure for ten, twenty, or even thirty minutes) (see p. 195). As a germicide, then, in watery solution, perchloride of mercury has a very high value. As an antiseptic it is of equal value, restraining the growth of bacteria when present in very minute proportion, even by 1 part in 50,000 (see p. 191). But in actual practice we rarely disinfect in pure watery solutions; the material to be treated is often pus, sputum, blood or other more or less albuminous substance, a fact which seriously detracts from the disinfectant activity of the perchloride. In Behring's often quoted experiments non-sporing

anthrax bacilli were killed, in a watery suspension, by 1 part of perchloride of mercury in 500,000; but in broth it required 1 part in 40,000, and in blood-serum, 1 part in 2000, to achieve the same result, indicating that the potency of the disinfectant was in inverse ratio to the amount of albuminous material present. Broth cultures of Staphylococcus pyogenes aureus may not be entirely killed, even in an hour or two, by a total strength of 1 in 1000 of the perchloride. Further, chlorides of sodium and potassium are commonly present in secretions requiring disinfection, and these, as has been already remarked, to some extent interfere with the germicidal action of the perchloride of mercury. We must therefore beware of taking the known disinfectant activity of the perchloride in pure watery solutions as a guide to what it can accomplish under the usual conditions in which disinfection is required. Misunderstanding on this point may create a false feeling of security in its use. Under suitable conditions, the mercuric salts are amongst the most powerful at our command, but they are unreliable in the presence of an albuminous or saline medium, unless used in greater strength or with more prolonged exposure than those commonly employed. Another matter requires to be again emphasised. It is a common practice to use the perchloride or biniodide of mercury dissolved in spirit instead of in water, as, for example, in the sterilisation of the skin, or of ligatures. Now these spirit solutions kill non-sporing bacteria in a few minutes, but they do so in virtue of the spirit they contain; plain spirit will kill such bacteria equally quickly. But when they are tested upon spores, which are not killed by the spirit itself,

their feebleness becomes manifest. Hay bacillus spores soaked for a fortnight in a 1 in 1000 spirit solution of biniodide of mercury are quite unaffected and grow well when transferred to nutrient broth; but in a watery solution of similar strength they are killed in a day or two. (See experiment on p. 195.) Krönig and Paul have shown that the disinfectant action of perchloride of mercury upon anthrax spores, though somewhat increased by the presence of a certain amount of alcohol (25 per cent.), grows less and less as the percentage of alcohol in the solvent fluid is increased, till, when the alcohol amounts to 80 or 90 per cent., it is practically abolished. The probable explanation is that hardly any free mercury "ions" are formed in alcoholic solutions of mercuric salts.* Other minor drawbacks to the use of the mercuric salts lie in their extremely poisonous nature and in their action upon metals. In poisoning by corrosive sublimate the best antidote to administer is white of egg, and this fact will serve to emphasise what has been said as to the effect of albuminous substances in checking its poisonous influence upon bacteria. As regards its action upon metals, it must be remembered that not only are instruments and other metallic objects spoiled by contact with the salts of mercury (which causes a precipitate of actual metallic mercury upon them), but also the disinfectant action is abolished in proportion as the mercury is precipitated.

**Carbolic Acid** and its Allies.—Carbolic acid or

* There are, however, certain *organic* combinations of mercury which are active in spirit solution. "Sublamin" appears to be such a compound: its true chemical name is "Ethylendiamin-mercuri-sulphate." Engels has recently shown that it is slightly more active in alcoholic than in watery solution.

"phenol" is used on a very large scale in practical disinfection, and it is therefore of great importance to know precisely what it can accomplish. In the intensity of its germicidal action it is much inferior to perchloride of mercury. It is not very soluble in water, so that a solution of 1 in 20 is the strongest that can be conveniently employed. It has little power of killing spores: in a strength of 1 in 20 it takes days to kill anthrax spores, and in a strength of 1 in 40 it will hardly kill them at all. Upon non-sporing bacteria its action is more vigorous; a solution of 1 in 20 kills them in a minute or so, in many cases in a few seconds (see p. 192). A solution of this strength is therefore adequate for surgical use, though not of much avail for complete and absolute disinfection. Carbolic acid has, moreover, two great advantages over perchloride of mercury. It is unaffected by metals, and can, therefore, be used for disinfecting instruments or for disinfection in metallic vessels. And its activity is little, if at all, impaired by the presence of albuminous substances. This renders it much superior to perchloride of mercury for disinfecting blood, pus, sputum, or fæces. Solutions of carbolic acid in alcohol or in oil have practically no disinfectant value whatever, save that alcohol itself kills non-sporing bacteria. The chief drawbacks to its use are its unpleasant effects upon the human skin when used in effective strength, and its more or less poisonous properties which have sometimes been manifested by absorption from extensive wounds. The statements made above as to the disinfectant action of carbolic acid apply to the chemically pure substance—*i.e.*, pure phenol. Much of what passes commercially

as "carbolic acid," contains only a percentage of phenol mixed with allied bodies such as "cresol." Such commercial brands, though probably less active than the pure substance, are yet valuable disinfectants, for cresol and the other bodies allied to phenol are not much less active than phenol itself. Mention may here be made of a substance known as *izal*, which has proved itself a useful disinfectant. It is prepared by distillation from coal-tar, and its precise chemical composition is roughly known; it appears to contain cresol and other bodies belonging to the phenol group, but not phenol itself. It is only slightly soluble in water, but forms a more or less enduring emulsion. It possesses disinfectant properties greater than those of pure carbolic acid, with the advantages that it is much less poisonous to man and less injurious to the human skin(see p. 193). It can thus be taken internally, and can be used as a gargle in efficient strength without the risk of deleterious effects. As a germicide against non-sporing bacteria it has much to recommend it, but against spores it is of little value as compared with the salts of mercury. A dilution of the strength of 1 part in 200 is suitable for ordinary use.

**Iodoform** is so widely used in surgical practice that it must be mentioned here. Its practical utility in the treatment of wounds and suppurations is undoubted, and it is therefore strange to find that, tested in the laboratory, it is almost devoid of disinfectant power. All observers are agreed on this point. The discrepancy between surgical experience and laboratory experiment has been explained by Behring as follows. In contact with decomposing discharges, the iodoform

is broken up into other and more soluble combinations of iodine. These are of greater antiseptic activity than the iodoform itself, and in particular tend to neutralise ptomaines and similar irritant products of decomposition. If this be so, the most that can be said for iodoform is that it is an antiseptic of a certain specific virtue in the treatment of wounds and tuberculous processes. No reliance must be placed upon it as a true disinfectant, especially apart from contact with discharging wounds.

**Formalin** is the name applied to a 40 per cent. solution of formaldehyd. It is of comparatively recent introduction as a disinfectant, and the proper fields for its undoubted activity are not yet precisely defined. The antiseptic action of formaldehyd is very great: in a strength of 1 in 5000 it prevents the growth of Staphylococcus pyogenes aureus, and that of many other pathogenic organisms in even greater dilution. In solution, its truly disinfectant action is less than might have been expected, and hence, as a liquid disinfectant, it has not come into general use. In the gaseous state, however, formaldehyd has shown itself a valuable and powerful disinfectant, and its powers will be discussed in the following lesson. A further objection to its use as an ordinary disinfectant lies in its chemical instability: its solutions undergo gradual decomposition and loss of power on keeping.

**Permanganate of Potash.**—The permanganates of potash and soda have been very largely employed as agents for destroying bacteria. The well-known "Condy's Fluid" owes its value to one of them. They cannot by themselves be classed amongst the more

powerful disinfectants, and they are not very strongly antiseptic. Miquel found that permanganate of potash acts as an antiseptic in a strength of 1 in 285, but strengths of 2 to 5 per cent. are required to destroy bacteria, and it would be unsafe to place reliance even on these for the destruction of spores. Nevertheless, Krönig and Paul found that a 4 per cent. solution of permanganate of potash killed anthrax spores in forty minutes. The permanganates have, however, a property which separates them somewhat from the substances which have hitherto been mentioned. They act as very powerful oxidising agents upon organic matter, readily parting with some of their oxygen, and thus helping to destroy the food upon which bacteria subsist. Their action is thus twofold. As pure disinfectants, they are not very powerful, but as a means of purging an organic fluid from a large proportion of its contained bacteria they are of great use by removing the chief material upon which the bacteria are able to multiply. It must not be forgotten that this very oxidising power is a hindrance to the true disinfecting influence of the permanganates in presence of the organic matter commonly present in material to be disinfected; for, as this is oxidised, the permanganate is used up and deprived of its powers. Permanganates have a very useful field of action as deodorants and as partial disinfectants, but they are not to be relied upon for absolute disinfection, save in combination with hydrochloric acid, or some equivalent salt, as will immediately be mentioned. In the cleansing of a foul water, a foul wound, or a foul cavity, such as the mouth, they have a very useful sphere of action in destroying the organic

matter which serves as a bacterial breeding-ground. Solutions of great strength, such as might be trusted to kill bacteria in reasonable time, possess unpleasant staining powers.

But although the permanganates themselves are not adapted for purposes of absolute disinfection, it has been found that their efficacy is immensely increased in the presence of hydrochloric acid. Krönig and Paul found that a 1 per cent. solution of permanganate of potash, containing one half per cent. of hydrochloric acid gas, was actually superior in germicidal power to a 5 per cent. solution of perchloride of mercury.* In such a mixture, the effective agent is not the permanganate, and not the hydrochloric acid, but a new compound which is formed—viz., hypochlorous acid—the substance to which bleaching powder owes its strong disinfecting powers. The mixture just named seems to be the most powerful agent at our command for the sterilisation of the skin in surgical practice. Another formula given by Krönig and Paul, and found by them almost equally efficacious, consists of 10 parts of permanganate of potash, $8\frac{1}{2}$ parts of common salt, and 25 parts of acid sulphate of potash, dissolved in 1000 parts of water. Neither of these solutions produces any injurious effect upon the skin, but they stain it a deep brown colour, which may afterwards be

* An actual mixture that will be found efficacious consists of 1 part of the permanganate and 1 part of the strongest liquid hydrochloric acid in 100 parts of water. In the writer's experience, anthrax spores, dried on the surface of smooth pebbles, are killed by this mixture in thirty seconds. But dried on silk threads it takes two or three hours to kill them, perhaps because they are protected by the deposit of manganese dioxide which occurs.

removed with a 1½ per cent. solution of oxalic acid.

**Bleaching Powder,** commonly known as "Chloride of Lime," is a very powerful disinfectant, largely used for domestic purposes. It is but sparingly soluble in water; its solution contains lime, and compounds of lime with hydrochloric and hypochlorous acids. The active disinfecting agent is the hypochlorous acid, but the alkalinity of the lime confers upon the solution certain important powers, notably the disintegration of such mucous material as sputum. For practical use no other chemical can compare with bleaching powder in the disinfection of sputum; carbolic acid and perchloride of mercury coagulate the sputa and hinder the access of the disinfectant to the central parts of the masses, but bleaching powder solutions break them up and dissolve them (see p. 213). It is also very useful for the disinfection of fæces. Nissen found that a solution of bleaching powder of the strength of 1 part in 500 killed Staphylococcus pyogenes aureus in one minute, while anthrax spores were destroyed in half an hour by a solution of 1 in 20, and in a little over an hour by a solution of 1 in 100. The familiar smell of "chloride of lime" is due to the hypochlorous acid liberated from it, and this constitutes one of the chief objections to its use. Its virtue deteriorates unless it is kept well sealed up, and it should preferably be freshly prepared. Its cheapness is of great advantage where large amounts of a disinfectant are required. The addition of a weak acid, even vinegar, greatly increases the disinfectant power of bleaching powder solutions, owing to the liberation of hypochlorous acid. In some experiments

carried out by the writer, a saturated solution of bleaching powder killed anthrax spores in ten minutes. Yet this same solution, diluted with twice its bulk of weak acetic acid (1·25 per cent.) killed the spores in less than a minute. But the addition of acids to solutions of chloride of lime causes the evolution of chlorine, which is then liable to attack metals.

**Boric Acid** (or Boracic Acid) is widely used in surgery, but its power of destroying bacteria is so feeble that even saturated solutions possess little disinfectant action. It is, however, a weak antiseptic and restrains bacterial growth, according to Miquel, in a strength of 1 in 143. Having little poisonous action, it has been largely used as a milk and food preservative.

* * * * *

The substances mentioned in the foregoing paragraphs are by no means all that may usefully be employed as antiseptics and disinfectants. But they comprise those which, having stood the tests of time and experience, are the best known and the most widely used.

The methods by which the activity of antiseptic and disinfectant action is practically tested in the laboratory are illustrated in the practical work described in the second part of this book.

# VII

## AËRIAL DISINFECTION AND THE STERILISATION OF FLUIDS BY FILTRATION

**Aërial Disinfection.**—Air itself can be mechanically freed from bacteria by filtering it through a layer of cotton-wool, and it is possible, by suitable mechanical contrivances, to pump into a room any desired amount of fresh air thus purified. Heating air to a sufficiently high temperature will also kill micro-organisms, but in practice this method has many disadvantages. Bacteria are carried down from the air mechanically by rain or snow, which exercise a marked effect in purifying it. This process may be imitated artificially by means of a fine spray. It must not be forgotten that in the undisturbed air of a quiet room the bacteria tend to settle down by gravity. Draughts and the dust raised by walking about hinder this process. The more dust there is in a room the more bacteria will be raised into the air by its disturbance. Wetting the walls and floor promotes the bacterial purity of the air, since the subsiding bacteria become adherent to the moist surface.

The problem of aërial disinfection, however, is less one of removing germs from the air itself than of using the air as a vehicle for the diffusion of gaseous dis-

infectants. After cases of infectious disease it is customary to "fumigate" a room with disinfectant substances, with the idea of destroying such bacteria as may be present in the air, or on the walls or other surfaces. There are three gaseous disinfectants in common use: sulphurous acid gas, chlorine, and formic aldehyd, and it is desirable to have some definite ideas as to their powers and limitations.

**Sulphurous Acid Gas** (Sulphur Dioxide) is usually generated by burning sulphur in the space to be disinfected.* Under the most favourable conditions as to the hermetical sealing of the room, and with the use of 1 or even 2 lb. of sulphur for every 1000 cubic feet of air, the amount of sulphurous acid gas which can be formed does not exceed 2 per cent., or thereabouts, of the room atmosphere. This amount cannot be breathed with impunity by man, and indeed when a room has been well "sulphured" after proper sealing, the air should be almost irrespirable, producing severe bronchial spasm, when the room is entered after twenty-four hours. Any animal left in the room will certainly be found dead if the operation has been properly carried out. But the death of higher animals is a very different thing from the death of bacteria. Under the most favourable conditions sulphurous acid is but an incomplete disinfectant. Spores are not destroyed even by prolonged exposure, but non-sporing bacteria in full

* Other methods are in use, *e.g.*, the combustion of bisulphide of carbon in a special lamp. The most powerful method is however the volatilisation of the gas liquefied under pressure. The liquefied gas is sold commercially and this method has recently been used for the destruction of rats in the holds of ships. It has the great advantage of being free from any risk of fire.

contact with the gas are in most cases killed. The difficulty is to ensure full contact. This condition is satisfied only in the case of bacteria lying absolutely on the surface of the articles in the room. The gas has very little power of penetration. Bacteria in articles of bedding or clothing, and those in the dirt of crevices, are apt to escape. It follows that, while the "sulphuring" of a room, properly carried out, may exercise a useful degree of superficial disinfection against non-sporing bacteria (to which the germs of most of our specific fevers belong), it is in no case to be relied on as a true disinfection, but requires to be supplemented by sterner measures. In the use of sulphurous acid gas for room disinfection it must be remembered that metal objects may be tarnished and vegetable dyes bleached. (For methods of testing gaseous disinfection see p. 200.)

**Chlorine Gas** is used in much the same way as sulphurous acid gas, and is generated from bleaching powder by the action of a mineral acid. The customary amount to use is 2 lb. of bleaching powder for every 1000 cubic feet of space in the room to be disinfected. Chlorine, at least in the presence of moisture, is a somewhat more powerful disinfecting agent than sulphurous acid, and it is to be noted that the activity of both these gases is intensified in the presence of moisture. The dry gases have little disinfectant power, but in the presence of water sulphurous and hypochlorous acids are formed respectively, and it is these acids which are the active chemical agents in disinfection. It is, however, subject to the same limitations, having little penetrating power, and the criticisms applied to sulphurous acid gas apply with much the same force to chlorine.

**Formic Aldehyd** (Formaldehyd or Formalin).—With the discovery of the powerful germicidal properties of this gas, it seemed likely that a new era in aërial disinfection had opened. Formic aldehyd is undoubtedly a far more potent gaseous disinfectant than either sulphurous acid or chlorine. Its vapour in reasonable concentration is capable even of killing spores, and under experimental conditions in the laboratory it fulfils all the requirements of a good gaseous disinfectant. The objections which attend its practical use are its expense and the difficulty of obtaining a sufficient concentration of its vapour in a good-sized room. If these objections can be overcome, formic aldehyd will undoubtedly replace chlorine and sulphurous acid gas as an aërial disinfectant. Various forms of apparatus have been already devised for the distribution of formic aldehyd, and of these one of the most recent, Lingner's glycoformal apparatus, appears to be extremely powerful. Yet, even with the most powerful apparatus, formic aldehyd seems to have only a little more penetrating power than chlorine and sulphurous acid, and cannot be relied upon for the complete disinfection of the contents of a room.

It follows from what has been stated that, in the present state of our powers, the sphere of aërial disinfection is limited. It is a very useful preliminary to the disinfection of a room after fevers, and renders the subsequent handling of the infected contents more free from danger. Ordinary "sulphuring," efficiently performed, should kill the less resistant forms of bacteria where they are superficially situated. The most powerful forms of formalin apparatus may kill

spores where superficially situated, but no reliance whatever can be placed on any known means of aërial disinfection for the sterilising of mattresses, bedding, clothing, carpets or curtains.

## STERILISATION BY FILTRATION

**Filtration** means the passage of a fluid, through a porous layer of some solid material, under the influence of pressure. The pressure must be higher on one side of the filter than on the other, or the fluid would not pass through. In ordinary filters the force of gravity is sufficient, but where the pores of the filter are very fine, artificial pressure has to be used—*e.g.*, forcible suction with a vacuum-pump or direct pressure with a force-pump.

It has been previously stated that a suitable layer of cotton-wool is an efficient bacterial filter for air; that is to say, the pores of the mass of wool are fine enough to prevent the passage of micro-organisms. We have here to consider the different materials used for the filtration of liquids, and especially of water, with the view of freeing them from bacteria. It is plain that the efficiency of any material in keeping back bacteria must depend upon the size of the pores in it; to be efficient the pores must be too small to let the bacteria through—*i.e.*, the material must be "germ tight." In a former lesson some idea has been given of the size of bacteria, and it will therefore readily be believed that not many substances which will let fluids through at all have pores small enough to restrain the passage of bacteria. Ordinary filter-paper or blotting-paper, for

example, hardly hinders their passage at all unless they are aggregated in clumps or adherent to grosser particles of organic matter. The turbidity of a broth culture of Staphylococcus pyogenes aureus is hardly altered by filtration, even through many folds of blotting-paper (see p. 202). And, indeed, there is no material capable of filtering water without the aid of artificial pressure, at least at a rate which can be of practical service, which will not let bacteria through. In other words, if its pores are fine enough to restrain bacteria, they are so fine that artificial pressure is required to get even water through at a reasonable rate.

The **domestic filter**, commonly constructed of carbon, spongy iron, or some porous earthy substance, and working by gravity, without artificial pressure, has, as a rule, no more influence in restraining bacteria than blotting-paper has (see p. 203). As a sterilising agent it is wholly ineffective. If new and clean, it may do some good by filtering off the grosser particles of organic matter present in a water, and with them doubtless many adherent micro-organisms. A water thus filtered becomes deprived of a part of that organic matter which serves as bacterial food, and hence is a less favourable breeding-ground. But no security whatever is afforded against the passage of bacteria, and unless very special care is expended in maintaining the cleanliness of the filter a serious danger is introduced. When such a coarsely porous filter has been in use for some time a large amount of organic matter clogs its pores, and the filter itself is thus liable to become a wholesale bacterial breeding-ground. After many months use it may thus come about that such a filter

positively adds bacteria to the water passing through it. Thus the *British Medical Journal* Commission (1894) found, in the case of a carbon block table filter which had been in use continuously for many months, that water which, before filtration, contained twenty or thirty germs per cubic centimetre, after filtration contained five or six thousand. It is plain that such filters are apt to become a delusion and indeed a snare, since they may create a false sense of security.

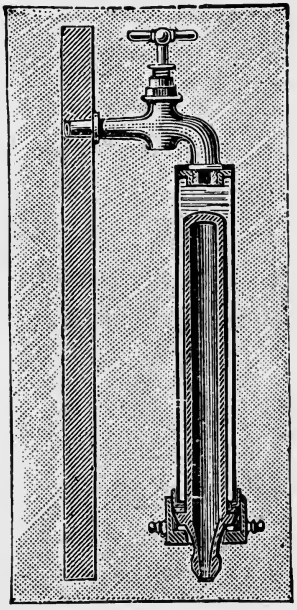

FIG. 20.—Sectional view of a small "Pasteur-Chamberland" filter, fitted on to a water tap. (Reproduced by the courtesy of Messrs. Baird and Tatlock.)

The filters which really filter off all bacteria from water are few in number and are all composed of a material so dense that artificial pressure is required for the passage of the fluid in reasonable time. And not even the very best will continue to deliver a sterile filtrate for any length of time: it is only a matter of a few days or weeks, and then the bacteria *grow* through the filter. The most reliable material for such filters is unglazed porcelain, as used in the *Pasteur-Chamberland* apparatus. The porcelain is made in the form of a hollow cylinder or "candle," closed at one end. The water passes from the outside of this, under the influence of sufficient pressure, into the central cavity and drips down into the collecting vessel placed beneath. For some little time the water passing through is absolutely deprived of germs, and

when, after some days, bacteria begin to appear in the filtrate, the filtering candle can readily be sterilised by careful boiling, after which it will again yield a germ-free fluid for a season. It should take three or four days for bacteria to grow through an ordinary Pasteur-Chamberland filter; for this length of time therefore the filter really sterilises (see p. 204). Denser forms of porcelain can be used which will yield a continuously sterile fluid for a much longer period, but the advantage gained is practically neutralised by the extreme slowness with which the water percolates such dense substances. Indeed the Pasteur-Chamberland filter is itself somewhat slow for ordinary use. In this respect the *Berkefeld filter* is its superior, while hardly inferior to it in efficiency. The Berkefeld filtering candle is similar in principle and form to the Pasteur, but is made of a compressed siliceous earth consisting of the flinty skeletons of "diatoms"—minute algæ which abound in salt and fresh water. This earth is found naturally in Germany and elsewhere, and is quarried and used for various purposes. A good Berkefeld candle should keep back all micro-organisms for three days, and it filters seven or eight times as quickly as the Pasteur candle. It has, however, this disadvantage, as compared with porcelain, that it is more friable and easily broken—a matter of some importance considering the frequent boilings necessary to keep it sterile. There are other filters designed on the same principles as those just mentioned, and some of them almost equally good.*

* An exhaustive series of experiments on domestic filters, carried out by Sims Woodhead and Cartwright Wood, will be found recorded in the *British Medical Journal* for November 10. 1894.

The subject of domestic water filtration may be summed up in the statements that only a few forms of high-pressure filter can be relied upon to furnish a truly sterile effluent, and that in these the filtering candle must be boiled twice a week if the effluent is to continue sterile. Those which continue efficient for longer periods filter so slowly as to be of little practical use. The pressure required for an ordinary Berkefeld or Pasteur filter is some fifteen or twenty pounds per square inch, and this is readily secured in an ordinary house by affixing the apparatus either to the rising main which supplies the cistern in the upper storey, or to a pipe at the bottom of the house descending from the cistern at the top. The method of sterilising the filtering candles by boiling is described in the practical work at the end of this book (p. 204).

The **filtration of public water supplies** on a large scale, as practised by water companies, is outside the scope of these lessons. It may, however, shortly be stated that it does not aim at the sterilisation of the water, but merely at the removal of the grosser particles of organic matter and at the reduction of the number of bacteria present. The water is filtered through beds of sand and gravel. These alone have pores far too coarse to arrest the passage of bacteria, but when they have been in use for a short time a deposit of organic matter occurs on their surface which is much more impervious and which does arrest the passage of bacteria to a considerable extent. It has been found practicable, by careful attention to the filtering-beds, to reduce the number of bacteria in a public water supply to less than 100 per cubic centimeter (or 56,000 per pint), and this is actually carried out in Berlin.

# VIII

## DISINFECTION IN SURGERY AND MIDWIFERY

In the foregoing lessons an attempt has been made to state the principal methods which are effective in the destruction of bacteria. In this and the following lessons it is proposed to deal with the practical application of these methods in Medicine and Surgery—not, indeed, in full detail, for that would be beyond the compass of an elementary work, but at least as regards the principles governing their use.

Modern surgery owes a vast debt to bacteriology, and it would be of infinite advantage to every surgeon and surgical nurse to have some practical acquaintance with bacteriological methods. Nothing short of ocular demonstration of what is and what is not real asepsis, and of how it can be attained, can carry absolute conviction.

The term *sepsis*, in its original Greek signification, meant "putrefaction," but nowadays, with the knowledge that putrefaction is exclusively associated with the presence of micro-organisms, sepsis has come to mean any undesirable form of bacterial infection or contamination. The main risks of surgery are now known to be those of sepsis, and modern surgery is essentially

concerned with its avoidance. When Lister first showed the mischievous part played by germs, surgeons began by placing great reliance upon chemical substances known to be noxious to them and capable of preventing their growth. Thus commenced the reign of *antiseptic surgery*, in which wounds were flooded with carbolic acid or other chemicals, and even the air of the operating theatre was impregnated with antiseptic sprays. But it was presently realised that antiseptics were harmful not only to bacteria but to the tissues of the human body. Their lesson had been taught, and experience soon showed that they might largely be dispensed with, provided that absolute cleanliness were secured before beginning an operation. Thus arose the era of *aseptic surgery* in which we now live. The essential principle of this is that, by the free use of disinfectant measures of every kind, the utmost attainable freedom from germs shall be secured before the operation commences. It has been found that, if no unsterile thing come into contact with the wound, it is needless to flush it with chemicals. All the advantages of antiseptic surgery are secured with none of its drawbacks. It is true that many a wound is, from the nature of the case, septic from the first, and here antiseptics may be needful, but this is commonly regarded as an evil necessity.

In the endeavour to secure effective surgical cleanliness, it is imperative in the first place to recognise the sources from which harmful bacterial contamination can arise. It has been pointed out in a previous lesson that only a very small proportion of the known species of bacteria can grow in the living body, or can interfere in any way with the healing of a wound. The dangerous

ones are those capable of setting up inflammation, suppuration, septicæmia, or other morbid process, and it is hence against these that the surgeon plans his campaign. Surgical disinfection is directed in the main against pus-forming bacteria, and especially against streptococci and staphylococci. It is an extremely fortunate thing that spore-bearing organisms are so rarely concerned in the untoward accidents of surgery. This should not prevent our aiming at their exclusion, but it renders a successful issue far more easy to attain.

At a surgical operation bacteria may gain access to the wound from the air, from water or other fluids used in irrigating it, from instruments, sponges, towels or ligatures employed, from the person or apparel of the surgeon, his assistants or nurses, or from the surface of the patient. Now the bacteria naturally present in fresh air are neither excessively numerous nor particularly harmful, and if the room or theatre in which the operation is conducted be clean and well ventilated, the risk of serious infection from this source may practically be neglected, especially as it is one against which it is scarcely possible to take effective steps. A moderate number of air bacteria are bound to settle in the wound in the course of an operation, but nearly the whole of these are, from their nature, incapable of growing there, and it is probable that the healthy tissues are fully capable of destroying the small remnant which might be capable of growth within the body. Very much the same is true of the ordinary bacteria in tap-water. These are nearly all harmless sorts, but they are far more numerous than air bacteria, and hence the risk of infection by water is greater. It is,

however, such a simple matter to obviate such risk as exists by boiling or effectively filtering the water, that this is always, and very properly, done.

The instruments, sponges, towels and other paraphernalia of operative surgery are not in their nature specially liable to harbour noxious germs, if we except catgut ligatures, which are made of essentially septic material. But they introduce a risk of a special kind: they may previously have been used in an operation on a septic case. This risk is so serious that the most jealous precautions are required in order to ensure their absolute sterility.

But the risk which overshadows all others is the risk of wound contamination from human sources, from the operator and his assistants, the nurses, and the patient himself. The bacteria which abound upon the human skin, in the human mouth, and in human clothing differ widely from those found in air and water, for a very large proportion of them, though living as saprophytes, are capable of parasitic existence, and they include all the principal pus-producing organisms. *Staphylococcus pyogenes aureus* and *albus*, *Streptococcus pyogenes*, *Diplococcus pneumoniæ*, *Bacillus coli communis* —these are the real enemies of the surgeon, and they all abound in and on the healthy human body. Here, then, is the real crux of aseptic surgery. Instruments can be boiled, towels can be steamed, and sponges can be soaked for weeks in strong disinfectants, but the sterilisation of the human body is far more difficult to secure, and can at the best be only superficial.

**Methods of securing Asepsis.**—One general principle deserves to be enforced at the outset. The

success of aseptic surgery depends upon minute and rigid attention to detail on the part of every one concerned in an operation, down to the humblest assistant. Neglect on the part of any one may wreck the whole operation.

In the next place it is to be observed that *hot water, soap, and a nail-brush* take the first place in surgical cleanliness. They may be supplemented, but never replaced, by chemical disinfectants. It is possible to remove nearly all bacteria from the skin by their use alone, for the chief abode of bacteria is not the living skin, but the layer of dead epidermis, dirt and grease on its surface. Hairy parts are more difficult to clean than others, so that shaving is requisite for a wide area around the site of an intended wound.

For the sterilisation of the instruments, towels, &c., heat should be employed wherever possible, for it is the surest, safest, and best means. **Instruments** are now commonly so constructed that they can be boiled without injury. Half an hour's boiling should destroy all pathogenic spores in the unlikely event of their presence, after which the instruments can be placed in an antiseptic solution till required. Steam disinfection is required for all cloths, towels, overalls, and similar textile fabrics. An abundant supply of sterilised towels should be at hand in an operating-room.

Where heat is inadmissible it is necessary to fall back on chemical disinfectants. The capacities of those in common use have already been described in a former lesson. For surgical purposes the perchloride and biniodide of mercury are the most generally useful, but carbolic acid has many points in its favour,

while izal is coming into favour with some. Each has its own advantages. For the final cleansing of the skin the combination of permanganate of potash with hydrochloric acid is the most powerful, and next in efficacy come the watery solutions of mercuric salts; for the cleansing of septic wounds carbolic acid is to be preferred to the salts of mercury. In choosing a solution for the reception of sterilised instruments a special consideration must be taken into account, namely, the effect of the disinfectant upon the human skin. Apart from the fact that the salts of mercury cannot be used for metal instruments, both perchloride of mercury and carbolic acid produce very unpleasant results upon the hands when these are wetted with them for hours at a time, as often happens. The skin is roughened and chapped, rendering thorough disinfection a much more difficult matter on subsequent occasions—a very serious thing for the surgeon. Izal possesses the advantage that it is less injurious to the skin than carbolic acid, while its superior as a surgical disinfectant. It has, however, the disadvantage that its dilutions are opaque, so that the assistant has to fumble for the instrument wanted. The water used for irrigation of wounds or cavities exposed in an operation should have been boiled. The water from an ordinary hot-water tap in connection with a boiler is sterile if the apparatus is in proper working order. It should be noted, however, that *physiological salt solution* is much superior to plain water for irrigating wounds, producing less injurious results upon the tissues. It should contain six parts of common salt (chloride of sodium) in a thousand parts of distilled

water. It is convenient to prepare and sterilise it in a strength three or four times greater than this and to dilute it, as required, with boiled distilled water. It need hardly be said that the vessels which contain these solutions must be sterilised as carefully as the fluid itself. The filtering of boiled water or salt solution before use through a porcelain or Berkefeld filter is a refinement which seems scarcely necessary, seeing that the fluid should be already sterile. It can only be required where the fluid contains gross organic particles—*i.e.*, where it was dirty before being sterilised.

The sterilisation of **ligatures** and of **sponges** is a matter of great importance and of some difficulty. Silk ligatures and those of silkworm gut and horsehair can easily be sterilised without damage by boiling, or, better, by steam disinfection. It is the catgut ligature which gives the trouble, for while the most generally useful, it is the most liable to be septic, and cannot be boiled in water or steamed without injury. Kangaroo tendon ligatures are in the same position. Two methods of sterilising them are possible. They may be boiled in some liquid which will not spoil them. Absolute alcohol is such a liquid, but its natural boiling-point is too low to ensure sterilisation; an apparatus is, however, in use in which the alcohol boils under pressure, whereby the boiling-point is raised sufficiently high for the purpose In America a liquid called "cumol," with a very high boiling-point, has been introduced with good result. The second method is a mechanical and chemical one. The catgut or tendon is scrubbed with a brush and soft-soap till it appears quite clean. The scrubbing should be carried on for half an hour or so. The

ligatures are then soaked successively in turpentine and in ether (five minutes in each) to remove all traces of fatty matter. They should then be sterilised by placing them for twenty-four hours in a 1 in 500 watery solution of perchloride or biniodide of mercury, or in a solution of similar strength made up in 25 per cent. spirit, but not in strong alcohol. There is no reason why they should not stop for several weeks in such solutions, but some surgeons prefer them stored in spirit. They should, of course, be untouched by hand from the time of their sterilisation till they are required for use. The process is lengthy and troublesome, but, if rigidly carried out, yields an aseptic ligature in good condition.

The dangers arising from sponges are so apparent that many surgeons will not use them, preferring "destructible sponges"—*i.e*, pads of absorbent gauze and cotton-wool, freshly prepared and sterilised for each operation. This is doubtless a safe position to take up, but such substitutes are inferior in absorbing power to real sponges. If sponges are used at all they must be used many times over, as they are too costly to be thrown away after a single operation. Some surgeons are willing to use again sponges which have been employed at an aseptic operation though rejecting those which have ever been in contact with pus or other septic material. It must be confessed that no absolutely reliable method of destroying spores in infected sponges, without injuring the texture of the sponges themselves, has yet been devised. They cannot be boiled or steamed, for this destroys their elasticity and power of absorption; reliance must therefore be placed on chemical

disinfectants. Even if spores are not certainly killed, non-sporing bacteria can easily be destroyed, and it is this which really matters to the surgeon, for the risk from spores may practically be neglected. In the opinion of the writer, it is legitimate to use marine sponges many times over, provided that proper precautions are taken in their cleansing and disinfection. These precautions must be considered under two separate headings:—(1) *Cleansing:* the great difficulty here is the coagulation of blood in the interstices of the sponge, whereby a network of fibrin is formed in its tissue, which it is very troublesome subsequently to remove. Sponges soaked in blood should not be allowed to lie about, but when done with should be squeezed out in water and put into a solution known to have the power of hindering coagulation, such as sodium oxalate, potassium citrate, or plain soap and water. The trouble from fibrin may thus be diminished. When the operation is over the sponges must be freed as perfectly as possible from all blood or organic matter which could serve as a breeding-ground for bacteria, or could hinder the subsequent action of the disinfectant. This is chiefly a mechanical affair. It is done by repeated washings and squeezings in strong washing-soda solution, followed by scrubbings with soap in running water till the sponge is apparently clean, when it is left to soak for twenty-four hours or more in strong washing-soda solution and again squeezed out several times. Nothing can replace the manual labour of this preliminary cleansing, which paves the way for the second part of the process. (2) *Disinfection:* for this it is plain that the most powerful solutions which will not

injure the sponges should be used.* A solution of sulphurous acid (1 part of the strong commercial acid to 3 of water) may be used. Such a solution further cleanses and bleaches, as well as sterilises the sponges, which should remain in it for twenty-four hours. Finally, after rinsing in boiled distilled water, they are stored till required in 1 in 20 carbolic acid solution. It is to be noted that in sterilising sponges and ligatures the hands should be most thoroughly cleansed and prepared as if for an operation, or they may convey to the sponge those very microbes which the surgeon has the best reason for dreading. A sponge which has been treated as above described is absolutely incapable of conveying to a wound the germs concerned in the production of suppuration or septicæmia. (The mode of testing the sterility of ligatures and sponges is described on p. 208.)

**The preparation of the surgeon** himself is all important, but he is, unfortunately, even more difficult to sterilise than a sponge or catgut ligature. The following remarks apply with equal force to his assistants, and in almost the same degree to the nurses attendant upon an operation. It is the business of the surgeon so to prepare his hands and arms that he may convey no germs to the tissues upon which he is operating, and so to clothe himself that risk of infection from this source is avoided. He must be careful that, during the operation, no particles are detached from his hair or beard; he should not speak loud or cough over the wound, lest his mouth prove a source of infection. He

* Bleaching powder solution cannot be used for the disinfection of sponges, as it disintegrates them.

must be scrupulously careful to touch no unsterile thing, and should be vigilant to ward off the access of anything of the sort from the wound. The ability to do all this can come only by careful attention to detail and long practice, but the necessary ritual becomes in time instinctive.

It is a very instructive thing to compare the bacterial flora of the hands in their ordinary condition, after scrubbing with soap and water, and after thorough preparation for an operation. Such an experiment is described in the practical work at the end of the book (p. 206). The skin bacteria are mostly in the dirty layer on the surface, but in smaller numbers they permeate the minute interstices between the epithelial scales for some little distance from the surface, and they are naturally prevalent in the crevices and inequalities about the nails. It is probably impossible to secure absolute sterility of the skin, but it is possible to secure complete sterility of its surface—that is, of such parts as will come in contact with the wound. Spores, it is true, cannot certainly be destroyed without destroying the skin itself, but the practical danger from such a source may be neglected. In actual practice the following precautions suffice: The nails must be closely trimmed and cleaned, and all foreign matter and epithelial *débris* removed from about their roots. The hands and forearms must be scrubbed with a sterile nail-brush, hot water and soap for ten minutes, changing the water several times. In this way the greasy layer on the surface should be so effectively removed that there is little need to use turpentine or ether after it, though this is sometimes done. Finally,

the arms and hands are to be well scrubbed and soaked in a strong disinfectant. The most powerful germicide available for this purpose is probably the mixture of permanganate of potash and hydrochloric acid mentioned on p. 99. The brown staining of the skin can be removed with a 1½ per cent. solution of oxalic acid. A watery solution of perchloride of mercury (1 in 500) is also of value. Spirit solutions of the perchloride or biniodide of mercury are in vogue with many surgeons, but, as has been already explained, their germicidal power is much inferior to that of watery solutions (see further, p. 197). Alcohol itself is a good disinfectant for non-sporing bacteria, and also helps to cleanse the skin from grease; but these advantages may be secured by a washing in alcohol alone, in addition to the use of the more powerful watery solution of the mercuric salt. The nail-brushes used in the scrubbing process should have been boiled in a 1 per cent. solution of carbonate of soda, and then preserved in a 1 in 20 solution of carbolic acid. Other good methods of sterilising the hands are in use, and may be found described in surgical text-books. All have the same object, namely, the complete removal of surface dirt, and with it the great majority of the bacteria, followed by the destruction of non-sporing bacteria in the surface layers of the epidermis. More than this it is futile to attempt; less than this is criminal neglect. By careful attention, true surface sterility can always be attained, as may be proved by bacteriological cultivations. Some surgeons, however, recognising the impossibility of attaining absolute disinfection of the hands, prefer to operate in thin rubber gloves,

the sterility of which can be assured by previous boiling.

In addition to the care of the hands, the surgeon should also have washed his face thoroughly, and if he wears a beard or moustache, special heed must be paid to the cleanliness of these. Very little attention is usually paid to the cleansing of the mouth; yet when it is remembered that the saliva contains a larger number of micro-organisms than the worst sewage, that streptococci and staphylococci are amongst the most numerous of these, and that they are proved to pass into the air in loud talking or coughing, it would appear worth the surgeon's while to take into account a cavity which comes so near the operation wound. Direct experiment proves that five minutes gargling with chlorine water, 2 per cent. solution of permanganate of potash, or 1 per cent. izal, will reduce the number of organisms in the saliva for more than an hour to something like 5 per cent. of their original number (Gordon). (For practical proof of these statements, see p. 209.) Lastly comes the question of the surgeon's raiment, and this seems satisfactorily settled by the adoption of a linen garment covering him completely from the neck to the feet. A supply of such overalls, previously subjected to steam sterilisation, should be at hand for all those taking part in the operation.

**The Preparation of the Patient.**—The principles here involved are in the main identical with those which concern the surgeon's hands. The skin, not merely at the seat of operation, but for a considerable distance around it, is to be rendered as aseptic as possible, in

order that when the incision is made no micro-organisms may be conveyed by the knife into the depth of the wound. The means employed are those already mentioned. The skin, if hairy, must be shaved and then mechanically cleansed with a nail-brush, soap, and hot water. Finally it is soaked with a disinfectant, but whereas in the case of the surgeon's hands this can only be applied for a few minutes, in the case of the patient's skin it can easily be applied for many hours by leaving on the surface a dressing saturated with the disinfectant, proportionately diluted, so as to cause no damage to the skin. Where the dressing is left on for six or eight hours, a suitable disinfectant solution is 1 in 2000 of perchloride of mercury. Operations which involve the mouth, rectum, or other septic cavity can be less perfectly dealt with by preliminary disinfection, but every measure should be taken to diminish the number of bacteria present. In the case of the mouth and pharynx, frequent gargling should be employed as a preliminary measure, chlorine water being as efficient a gargle as any, while due attention should be paid to the condition of the teeth. In the case of the rectum, purgatives and enemata are of service, with a final disinfectant enema of izal or permanganate of potash, retained as long as possible. The risk of poisoning by absorption from the rectum must be borne in mind. In operations involving the intestinal tract, such internal disinfection as is practicable should be previously carried out. The intestinal disinfectants which have been shown experimentally to be of service in diminishing the number of bacteria in the fæces are salol, izal, and urotropin, but preliminary purgation is of great importance.

**Dressings.**—Gauze or cotton-wool dressings may be either antiseptic or aseptic. A simple aseptic dressing which has been subjected to steam disinfection and then carefully preserved from contact with the air till required should be perfectly satisfactory for all aseptic wounds; but where the wound is septic, or may be expected to become so, or where a large amount of discharge is anticipated, antiseptic dressings are indicated. The amount of disinfectant present in most dressings is not sufficient to sterilise septic discharges absorbed into them, but it may be enough to restrain bacterial growth and so be of some service. It is not superfluous to add that care should be taken that antiseptic dressings are also aseptic. Dry dressings may be contaminated by dry bacteria from the air, from dust, or in handling, and the antiseptic present will have no influence at all; it requires moisture to bring it into solution before it can act. Antiseptic dressings should therefore undergo steam disinfection, just as aseptic dressings do, before they are used; in other words, all dressings should be aseptic, while in many cases it is also desirable that they should contain a chemical disinfectant. (For testing the sterility of dressings, see p. 209.) In the choice of an antiseptic dressing it is well to bear in mind that the albuminous discharges from wounds greatly hinder the disinfectant action of the salts of mercury.

**Drainage-tubes and other Indiarubber Materials** used in surgical operations may be sterilised by boiling, and then preserved for use in 1 in 20 carbolic acid. In conclusion, it is scarcely necessary to say that on every occasion on which a wound is

dressed precautions must be taken to prevent its contamination similar in principle to those which have just been discussed.

**Disinfection in Midwifery.**—All that has been said concerning the importance of asepsis in surgery applies, in general principle and with equal force, to the conduct of labour. The service which bacteriology has rendered to surgery has been rendered in almost equal degree to obstetrics, for sepsis is also the greatest risk of childbirth. The application of those very principles which enable the surgeon to open the peritoneal cavity with impunity has practically done away with this great risk. Puerperal fever still occurs, it is true, but its causes are recognised and its spread can be checked.

After the birth of a child the site of attachment of the placenta and the lacerated passages through which the fœtus has passed constitute what is to all intents and purposes an immense internal wound, which has to heal before it is safe from infection. Under natural conditions of birth, such as prevail amongst the lower animals, this risk is very slight, precisely because the wound is an internal one; it is only at the external opening of the genital passage that infection is possible, and experience shows that it is here little liable to occur. The chief point of danger is the raw internal surface of the uterus, and under the artificial conditions which civilisation has brought about, it is the manipulations of the midwife which are the source of risk. It is the introduction of the hand or of instruments into the genital passages during or after labour that carries with it the risk of introducing pathogenic organisms.

The bacteria which can do harm are precisely those which do harm in surgery. They are especially the pus-producing cocci, and above all streptococci. The vast majority of cases of puerperal fever are due to the streptococcus pyogenes, which is pre-eminently, though doubtless not exclusively, the cause of this dangerous disease.

The guiding principle of modern midwifery is thus simple and identical with that obtaining in modern surgery: it is "asepsis, combined with antisepsis when needful." The rules by which the principle can be carried out are equally plain. Nothing is ever to be introduced needlessly into the genital passages, and the hand in particular should not be needlessly introduced, because it is less easy to disinfect than an instrument. Examination by the finger is in most cases requisite to ascertain how the case is progressing, but such examination should be recognised as a necessary evil and should be as far as possible avoided, just as a surgeon would avoid the unnecessary introduction of his hand into the peritoneal cavity. The hand introduced should be sterilised as carefully and in the same way as the hand of a surgeon who is about to perform a severe abdominal operation, and the accoucheur or midwife should be as careful as regards clothing and other details. In the event of instrumental interference, the instruments should be as elaborately sterilised beforehand as at a surgical operation. In the preparation of the patient for a confinement, the only measure of asepsis which is as a rule necessary is the thorough cleansing of the external genitals with soap and water, followed by 1 in 1000 perchloride of mercury, or

biniodide of mercury solution. No attempt at disinfection of the vaginal canal is needful in a healthy woman; it is, indeed, probable that the vaginal secretions themselves possess some germicidal properties. The exception to this rule is where gonorrhœal infection exists: in such a case, in the interests of the child, it is desirable to cleanse and disinfect the genital passages as far as possible, on account of the danger of gonorrhœal ophthalmia. A thorough douching with carbolic acid solution is the usual measure advised in such cases, but the stronger solutions are unsafe, as absorption may occur. A strength of 1 in 40 is the most that can be used, and many prefer 1 in 80. The germicidal powers of the latter are somewhat feeble, but thorough douching even with plain warm water must remove the greater part of the infective material present. It is possible that the far less poisonous izal may prove useful in this field. Another question in this connection concerns the administration of antiseptic douches after labour is ended. The surroundings of the patient must here be taken into account. If these are septic and unclean, the risk of introducing micro-organisms in the process of douching is commonly held to outweigh the advantages of the douche; but in hospitals and in private houses of the better class, where reasonable asepsis is assured, the risk is so slight that the balance of advantage is in favour of the douche. In gonorrhœal cases, and in cases which have already become septic, disinfectant douches have naturally a wide field of usefulness, but space fails for their further discussion here. The principles which should underlie their use have been sufficiently emphasised in the fore-

going lessons. The only further point which may be mentioned in this place is the attention which must be paid to the eyes of the infant where the mother is suffering from gonorrhœa. The chief measure requisite is a thorough flushing of the conjunctivæ in all their recesses with warm water, followed by 1 in 1000 solution of perchloride of mercury, and this again by sterile warm water.

# IX

## DISINFECTION IN MEDICAL CASES; with a short Account of some of the Bacteria concerned in Infection

THE mystery which, in former ages, enshrouded the spread of infectious disease is now in great part abolished. We no longer look upon infection as a vague exhalation from the patient or from those who have been in contact with him. It is an accepted doctrine that all infectious diseases are due to micro-organisms, and that the mysterious something which leaves the patient's body and causes the disease in others is a concrete living microbe. It is true that this is not absolutely proved in detail for every infectious disease, but it is completely proved in many and partially proved in others. There are still some fevers in which the causal agent has eluded detection, but we believe these also to be due to micro-organisms, because it is incredible that a poison which is capable of indefinite multiplication should be other than a living organism. It is the future task of bacteriology to trace the nature of these unknown microbes, as it has traced those already known.

But bacteriology has done more than merely indicate the nature of infection. It has enabled us in many

cases to determine with exactness the paths by which the infecting agent leaves the body, and the facts which have been brought to light have sometimes been as important as they were unsuspected. The occasional persistence of diphtheria bacilli in the throat during convalescence, and the frequent occurrence of typhoid bacilli in the urine and sputum are cases in point. Speaking generally, it may be stated that infection leaves the body by the mouth (throat secretions, sputum, &c.), by the nose (nasal discharges), by the skin, in the urine, or in the fæces. Occasionally it may pass out by other channels, as from the conjunctivæ, or from wounds or suppurating areas. The path of escape varies in different diseases, according to the primary seat of infection, and the liability to the spread of the microbe in the body. An attempt will be made, in the pages which follow, to indicate the special risks attending each of the principal infectious diseases of man. It has already been pointed out in the third lesson that the power of parasitic bacteria for evil depends largely upon their capacity for resistance outside the animal body. Those which easily die on drying can only infect in the immediate vicinity of the patient. Those which can withstand desiccation may be conveyed by the air for long distances, or by the intermediation of those who have been in contact with the sick. Those which can grow as saprophytes outside the body have still wider potentialities for evil.

Persons who come into continuous contact with cases of infectious disease, and, in particular, nurses who are daily and hourly exposed to direct infection, are of necessity specially liable to become themselves infected.

The duty of a nurse in attendance upon an infectious case is twofold. She must in her own interests take all possible means to minimise the risk of her own infection, and she is further responsible for the carrying out of such measures of disinfection as can be devised for preventing the spread of the disease to others. In their essence, the details of nursing infectious cases, apart from their medical aspect, are simply bacteriological problems, and will here be considered chiefly from that point of view. We have first to consider the general precautions advisable in all infectious cases, and then the special precautions requisite in the individual fevers. It is of the greatest service to have all possible knowledge as to the precise nature of the infecting agent in any given case, and as to its degree of resistance to disinfectant agencies.

**The Sick-room.**—In fever hospitals special wards are as far as possible set apart for the different fevers received. In general hospitals special infectious wards are, as a rule, provided: it is never desirable that cases of the specific fevers should be treated in the general wards, though in the case of typhoid the risk of doing so is less than in most other fevers. But it is with the nursing of infectious cases in private houses that we have here chiefly to deal. The room selected as a sick-room should be as remote as possible from other inhabited rooms, and, where feasible, it should be at the top of the house, because the air currents in a house are mostly upward currents; thus the risks of aërial infection are diminished. The room should be of good size, in order that infectious material present in the air may be as much diluted as possible, and it should be

capable of free ventilation for similar reasons. The windows should be large and should not have a north aspect, because much sunshine is desirable. Sunlight and fresh air are amongst the most important agents in disinfection. Bearing in mind the disinfectant measures which will have to be taken at the close of the case, all superfluous furniture should be removed beforehand, especially articles of any value. Curtains and carpets are unnecessary. The room should have a good fireplace, and, except in intolerably hot weather, it is well to keep a fire burning, for it is a useful aid to ventilation and serves for the ready cremation of infectious material. It is a common practice to place in the sick-room shallow vessels containing "disinfectants": it need hardly be said that, as a measure of disinfection, such a proceeding is absolutely futile; any efficient means of aërial disinfection would assuredly kill the patient long before it caused the slightest qualm to any microbe. Such substances as eucalyptus oil or sanitas are, however, agreeable deodorants where the need for such exists. Again, it is a common practice to hang outside the doorway a sheet soaked in weak carbolic acid. This, too, is perfectly useless as a measure of disinfection: the only thing it can do, so long as the sheet is wet, is to arrest some of the particles in the air, and so far it may be useful. The practice, however, is not one to be discouraged, for the sheet is an admirable fetish, proclaiming to all who approach the infectious nature of the malady, and bidding them beware. A very useful measure is the placing of a foot-bath or similar vessel filled with 1 in 40 carbolic acid solution, just outside the door of the sick-room.

In this can be placed all the cups, plates, spoons and such-like articles which have been used by the patient or nurse before they are taken downstairs to be washed up. A similar, but larger, receptacle may also receive sheets, pillow-cases, handkerchiefs, and other articles, which should remain soaking in the disinfectant for twenty-four hours before going to the laundry.

**The Nurse.**—In the selection of a nurse for an infectious case, attention should be paid to her age, general health, and previous illnesses, in addition to her professional qualifications. Speaking generally, the younger a person is, the more liable is she to infectious disease; a nurse of middle age will be less liable to contract infection than a younger woman. Feeble health is a predisposing cause to bacterial infection, so that only the strong and healthy should be employed to nurse infectious cases. If possible, a nurse should be found who has already suffered from the fever in question, provided always that she has not already suffered from it several times, indicating a special liability to the particular disease. In the case of small-pox, recent revaccination is a necessity.

While in attendance upon the case she should pay due attention to her own health. She must be liberally fed, and should take fresh air and exercise daily, while due provision must be made for a daily bath. She must have adequate time for sleep, so that it is needful to have both a day and a night nurse for a case requiring much attention. The nurse's sleeping-room should adjoin the sick-room, so that she may be readily summoned if required, and this room is also to be regarded as an infectious room, not to be entered by others.

Before going out for her daily exercise she must wash, or bathe, and change her dress for one never used in the sick-room itself, and she ought not to travel inside a public conveyance. She should, in fact, regard herself as, in a measure, an outcast, and must not mingle with others in the house. Her dress must be of a washing material capable of easy disinfection. At the close of the case, before returning to ordinary life, her clothes and effects will require disinfection like the rest of the appurtenances of the sick-room, and she must further disinfect her person before assuming her non-infected attire. To this end a thorough scrubbing from head to foot with hot water and soap is the main thing. A woman's hair presents the chief difficulty in this respect, and it is advisable, in addition to the washing, to soak it well in carbolic solution of not less strength than 1 in 40, and preferably stronger.

**General Precautions in the Nursing of Infectious Disease.**—Strict *isolation* is of the first importance, and this rule can only be relaxed with safety in the case of diseases such as typhoid or septicæmia, in which aërial infection seems to play little or no part. In typhus, small-pox, chicken-pox, scarlet fever, measles, rubella, mumps, whooping-cough and influenza, isolation should be of the strictest. Where it is a matter of urgent importance that relatives or friends should be admitted to the sick-room, they should observe the same precautions on leaving it as have been mentioned in the case of the nurse. It is a good plan to provide easily sterilised, capacious linen overalls reaching from the chin to the feet and completely covering the ordinary clothes. These may be donned by visitors and by the

doctor outside the door of the room and taken off after the visit.

**Infected linen and clothing** may, in the majority of cases, be soaked for twenty-four hours in an efficient disinfectant solution (carbolic acid 1 in 20, izal 1 in 100) and then sent to the wash in the ordinary way. The articles must be entirely submersed in the fluid, not merely wetted with it. This treatment obviates all risk in typhoid, diphtheria, cholera and influenza, and probably also in scarlet fever and most other zymotic diseases. But there is one fever at least in which such a measure may be insufficient—namely, small-pox, for there is some probability that this is due to a spore-bearing bacillus. Failing access to a steam-disinfecting apparatus, linen and clothing from a small-pox case should either be boiled in a cauldron for half an hour, or soaked for twenty-four hours in a perchloride or biniodide of mercury solution (1 in 1000); preferably it should be boiled. But in most towns the local sanitary authority possesses a steam disinfector, and will be ready to undertake the sterilising of infected linen. In all infectious cases such an arrangement will save trouble and gives the highest attainable security against the spread of infection.

**The excreta and discharges of the patient** should in most cases be subjected to disinfection; and, indeed, to be on the safe side, this should always be done. It is true that only in a few fevers, such as typhoid and cholera, do we know the fæces to be eminently infectious, but the urine, sputum and throat secretions may contain the specific micro-organisms of the different fevers far more often than is commonly sup-

posed. The *stools* of infectious cases are the most difficult to disinfect. They should be mixed with more than an equal bulk of 1 in 20 carbolic, 1 in 50 izal, or 1 in 50 chloride of lime and well broken up with a stick or iron rod (which must be subsequently burned or heated in the fire) so that no lumps remain. Perchloride of mercury is not to be recommended. The stools must remain in contact with the disinfectant for two or three hours before being poured away down the water-closet. The *urine* may be mixed with an equal volume of one of the solutions just named for an hour or so before it is poured away. *Sputum*, when copious, may be similarly treated, but when scanty is best put in the fire. The most reliable disinfectant for sputum is a solution of fresh chloride of lime (1 in 50 or 1 in 100). The *secretions from the nose and throat* should be received on rags or cotton-wool and burnt at once. The cheap Japanese pocket-handkerchiefs made of thin, tough paper are very serviceable for this purpose. It is sufficient to place the *cups*, *plates*, *spoons* and other vessels and utensils used by the patient, in a solution of carbolic acid (1 in 20) or izal (1 in 100) for an hour or so before they are washed up; or they may be scalded with boiling water.

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It now remains to discuss the different common infectious diseases with regard to the special indications for disinfectant measures in each.

**Small-pox** (or Variola) stands, so far as we know, in a somewhat different category from the other specific fevers in two respects. One of these is that we possess in vaccination and revaccination

a means of protection which experience has shown to be very complete. Somewhat analogous proceedings have been devised in the case of a few other fevers (typhoid, plague and cholera), but the protection conferred does not approach in completeness to that given by vaccination against small-pox. It is necessary for those coming into contact with small-pox cases to be revaccinated, for it is well established that protection gradually fades. No one need object to be revaccinated, for if it is not required it will not take. It is important to remember that vaccination is effective in preventing the development of small-pox, even if performed two, or probably three days after exposure to infection. After a longer interval than this it is ineffective. The second point of importance concerning small-pox is that there is some ground for supposing it to be due to infection with a sporing organism, which is not known to be the case in any other fever. The evidence, it is true, is by no means complete, but two independent observers (Klein and Copeman) have shown the presence of a minute spore-bearing bacillus in the early stages of the disease, while all experience goes to prove that small-pox infection is more persistent and difficult to destroy than that of most other fevers. It is, therefore, a wise thing, in cases of small-pox, to insist on measures of "complete" disinfection—*i.e.*, such as will ensure the destruction of spores. Heat—wherever possible in the form of steam disinfection—is therefore to be the main agency upon which reliance should be placed. Where this is impossible, infected material should be steeped in solutions of perchloride or biniodide of mercury not weaker than 1 part in 1000, and for no shorter time

than twenty-four hours. The rule that visitors to small-pox patients should wear linen overalls must be rigidly enforced. Although it is probable that all the excretions of a case of small-pox may convey infection, yet the main source of danger lies in the secretions and crusts from the pustules on the skin. The breath can only convey the disease to those in the immediate vicinity of the patient, but the dried epithelial *débris* from the skin may be carried in the air for long distances. It is well established that small-pox hospitals may serve as centres of infection when situated in thickly populated districts, such influence extending even for a mile, and the air appearing the only possible channel of dissemination. Under these circumstances it is plain that it must be of great importance in the nursing of small-pox cases to check as far as possible the escape of particles into the air from the patient's skin. The most effective means of doing this is to keep the skin moist with some greasy or sticky preparation, such as oil, lanolin, or glycerin solution. Detached crusts should be cremated. Undisinfected clothing and bedding may retain infection for long periods.

**Chicken-pox** (Varicella).—The nature of the infecting agent in this complaint is unknown, but it may be conjectured, from its analogy, in some respects, with small-pox, to be of a somewhat similar nature. Precautions as to disinfection should therefore be directed against a possibly spore-forming organism, and attention should be paid to the skin, as in small-pox.

**Scarlet Fever** (Scarlatina).—There is a presumption, and a fairly strong presumption, that the infective agent in this disease is a streptococcus, and that the

seat of primary infection is the throat. The proof of this is as yet incomplete. It has been shown experimentally that the secretions from the throat are capable of conveying the disease to healthy human beings. Klein proved that an infective disease amongst cows, which was associated with a widespread epidemic of scarlet fever amongst the consumers of their milk, was due to a streptococcus, and Klein and Gordon have since shown that this organism (Streptococcus scarlatinæ of Klein) is constantly demonstrable in the throats of scarlet fever cases and not in healthy throats. There is thus some ground for assuming that the infective agent in this disease is one which forms no spores; but, in the absence of proof that pure cultures of this streptococcus can cause scarlet fever in the human subject, final judgment must be suspended, and it is safe to place reliance only on disinfectant measures which can destroy spores. The evidence is, however, enough to warrant us in laying great stress on the danger of infection from the throat secretions in scarlet fever, and this even in the earliest stages of the disease. There is an old superstition that little danger of infection is at first present, and that the risk is greatest when the patient begins to peel. So far as direct evidence goes, this view is diametrically opposed to facts. There is no bacteriological evidence of the infectivity of the desquamating skin, and the clinical evidence might fairly well be explained by persistence of the infecting agent in the throat. It would be by no means right to assume that the desquamated particles of the skin are devoid of infection, but modern opinion is gradually coming to lay more stress on the

danger of the throat secretions and less on the peeling epidermis. Apart from direct personal contact with the patient, infection may be retained in clothing and bedding for some considerable time, and may convey the disease to others. There is, however, no evidence that scarlet fever can "strike" through the air for long distances, as has been proved to be the case in small-pox. In certain cases a convalescent patient, in whom even peeling has ceased, may apparently infect others; of such a nature are the "return cases" which occasionally occur in households receiving patients discharged from fever hospitals. It is possible that here the infecting agent has persisted in the throat after convalescence. Disinfectant measures in scarlet fever must be specially directed against the secretions of the throat. Kissing must be absolutely prohibited. All discharges from the mouth and nose, and also those from the ear when this is affected, must be received on rags or thin paper handkerchiefs and burned. The seat of infection may also be directly attacked by the use of disinfectant gargles, of which chlorine-water is one of the best. During desquamation, attention must be paid to the skin to prevent, as far as possible, dissemination of the particles through the air. This may be attained in large part by keeping the skin oiled, and by occasional baths followed by ablutions with warm 1 in 40 carbolic solution. The precautions as regards disinfection of linen, utensils, &c., do not differ from those applicable to all infectious fevers. The urine may contain the specific microbe and should always be disinfected.

**Diphtheria.**—In this disease we have to deal with a known infecting agent, the Klebs-Löffler bacillus.

This bacillus does not form spores, and can be destroyed by all those agencies which kill non-sporing organisms. It is killed by exposure to 140° F. for ten minutes, and has no special powers of resistance against chemical disinfectants. It can, however, withstand drying for many weeks. The common seats of infection are the throat and upper air-passages, and the infection is local. It may spread over the mucous membranes by direct extension, but the bacilli do not habitually invade the blood or reach distant organs. The risk of infection in diphtheria (apart from the rare cases in which the disease attacks other mucous surfaces) is thus essentially a risk arising from the secretions of the throat, nose and air-passages generally, but infection may be conveyed by linen, clothes, &c. upon which such secretions have dried. Two special points must be mentioned in this connection. The first concerns the use of antitoxin. Before the introduction of this valuable remedy, the chief means of combating the disease lay in the application of disinfectants to the throat or diseased mucous membranes. There is danger lest, with the use of the new remedy, the value of the old should be overlooked. Diphtheria antitoxin, as usually prepared, acts as an antidote to the poison formed by the diphtheria bacillus, and does great good; but it has little germicidal action upon the bacilli themselves, which may continue to flourish in the throat though their evil effects are antagonised. It is therefore as needful as ever to apply local disinfectants to the seat of disease. The second

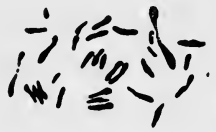

FIG. 21.—Diphtheria bacilli, magnified 1000 diameters. (Drawn from a micro-photograph of a stained preparation.)

point is that, while in most cases the diphtheria bacilli vanish from the throat within a week or a fortnight from the time the membrane has disappeared, there are other cases in which they persist much longer. Occasionally they may be found by cultivation for months after the disease has gone: in one case the writer has found them present, still virulent in their effect upon animals, five months after the attack of diphtheria, and even longer periods of persistence have been recorded. Fortunately such long persistence is far from common, but it emphasises the rule that no case of diphtheria is to be regarded as free from the danger of conveying infection until cultivations from the throat have been found free from diphtheria bacilli—a most vexatious rule for victims who are in perfect health, but one from which it is never safe to depart. It is to be noted that, when a culture is to be taken from the throat, no disinfectant should have been applied to it for at least twenty-four hours. As regards the measures to be taken for the local disinfection of the throat, the following have proved of service: (1) Painting the affected parts with a solution of perchloride of mercury of a strength of 1 in 500: fifteen or twenty minims of such a solution may safely be used for a single application, as they do not contain more than the medicinal dose of the drug. The albuminous matters present hinder the action of the perchloride, but there is this advantage in the treatment, that the mercury, swallowed and absorbed, is to some extent re-excreted in the saliva, keeping up a mild mercurial bath for the fauces which one may suppose to be inimical to the diphtheria bacilli. (2) Various gargles or sprays may be employed: of these

the best are chlorine-water, permanganate of potash solution (1 in 300), and izal (1 in 100, or even stronger, if the patient can bear it). Formalin in 1 or even 2 per cent. solution may be tried, and peroxide of hydrogen has at times seemed of use. In cases where the bacilli persist for long in the throat it is well to use these methods successively, each for a few days, in the hope that, if the bacilli become inured to one drug they may succumb to another. The great difficulty in such cases is the impossibility of reaching all the mucous surfaces of the upper respiratory tract with adequate disinfectants. Nasal douches may be used to supplement gargles, but even thus the accessory sinuses of the nose are not reached. In the nursing of diphtheria cases it is of special importance that the nurse should avoid inhaling material coughed up by the patient. But in a bad tracheotomy case the nurse or medical man cannot always avoid this, and it is advisable that those attending on cases of diphtheria should systematically gargle twice a day with chlorine-water or other good disinfectant, in order to diminish the risk of infection. Where this risk is very great it is a wise thing to submit to a prophylactic injection of diphtheria antitoxin (500 units): the immunity thus conferred lasts three or four weeks. As in scarlet fever, the throat and nose discharges from the patient should be received on rags and burnt. Membrane required for bacteriological examination should be seized with sterile (recently heated) forceps and placed in recently boiled and cooled water in a sterilised test-tube, which should then be plugged with cotton-wool. The other precautions with regard to diphtheria do not differ from

those to be employed in the infectious fevers generally. It is, however, to be noted that some of the lower animals, and especially cats, are liable to infection, and may spread the disease. Cats should hence be carefully excluded from the sick-room.

**Measles, Rubella, Influenza** and **Whooping-cough** are diseases which may be considered together, for the reason that the primary seat of infection in all is probably the respiratory tract. In the case of influenza the infecting agent appears to be the minute bacillus described by Pfeiffer and Canon, though absolute proof of this is wanting. This bacillus is not known to form spores. In the other three diseases there is no sufficient proof as to the nature of the infecting agent. In none of them is the infection of a very resistant or lasting nature, though it may persist for some weeks in the person of the patient, especially in the case of whooping-cough. It may, therefore, be conjectured with some probability that it is not, in either of these diseases, a spore-bearing organism. The only special point in connection with the nursing of these cases is the necessity for disinfection of sputum and discharges from the mouth and nose. In measles, discharges from the eyes may require similar attention.

**Mumps** (or Epidemic Parotitis).—This is an eminently infectious complaint, in which the infective agent has not yet been certainly identified. From the habitual site of the affection, in the salivary glands, it may be presumed that infection leaves the body by the mouth secretions, but this is merely a presumption. There is nothing to show whether the infecting agent forms spores or not.

**Epidemic Cerebro-spinal Meningitis** is an obscure disease. It is almost certain that its cause is infection by a coccus, which forms no spores—the "Diplococcus intracellularis" of Weichselbaum—but we are in complete ignorance as to how infection gains access to the body and how it leaves it. It is not even certain that infection can be directly conveyed from the sick to the healthy. Reliance can therefore be placed only on general measures of disinfection.

FIG. 22.—Pneumococci, in pairs, showing the faintly stained capsules by which they are surrounded. (Magnification 1000 diameters. Drawn from a stained preparation.)

**Acute Pneumonia.**—This disease is often classed as an acute specific fever. But it is rather a group of diseases. The inflammation of the lung, which is its cardinal feature, is most commonly due to the pneumococcus or "Diplococcus pneumoniæ," which is present in great numbers in the sputum. This organism forms no spores, and is destroyed with great ease outside the body. It is present in the secretions of the respiratory tract in the majority of healthy persons. It follows that the "causes" of this, the common form of acute pneumonia, are to be sought, not in infection from without so much as in a lowering of the resistance of the tissues of the lung, so that the disinfectant measures requisite in the true infectious fevers seem here uncalled for. Nevertheless, cases in which the disease has appeared infectious are not absolutely unknown. This is perhaps explained by the fact that other forms of the disease occur in which the infective agent is not the ubiquitous pneumococcus, but a more truly specific organism. In influenza and in plague, infection may

at times manifest itself in the form of a primary pneumonia. And there have occurred outbreaks of infectious pneumonia, as in the well-known Middlesborough epidemic, in which a definite micro-organism —the "Bacillus pneumoniæ" of Klein, allied to Bacillus coli communis—has appeared to be the exciting cause. In such visitations of epidemic and infectious pneumonia disinfectant measures are plainly indicated, and the sputum is evidently the chief secretion to disinfect.

FIG. 23. — Typhoid bacilli, stained by the silver method to show their numerous flagella, or organs of locomotion. (Drawn, somewhat idealised, from a microphotograph: magnification, 1000 diameters.)

**Typhus Fever** is a disease the bacteriology of which is at present unknown. It is certain that it is intensely infectious to those in the immediate vicinity of the patient, but it is also well established that the poison is easily destroyed by fresh air and ventilation, from which it may be inferred that it is probably not a spore-forming organism. Infection may be retained for some time in clothing or bedding. In **relapsing fever** the cause is almost certainly a Spirillum, found in the blood during the periods of fever. This has never been cultivated, but there is no evidence that it forms spores. Neither in typhus nor relapsing fever are there any special indications for disinfectant treatment other than those common to all infectious fevers. Free ventilation must be insisted on.

**Typhoid Fever** (Enteric Fever).—The infecting agent is here well known. It is a motile bacillus known as Eberth's bacillus or the Bacillus typhosus. It forms no spores, is killed in ten minutes at 133° F., and is one

of those organisms most easily destroyed by chemical disinfectants. It is capable of living as a saprophyte outside the body for some time, and epidemics are brought about as a rule through polluted water or articles of food—*i.e.*, by indirect and not direct transfer from the sick to the healthy. Direct infection is, however, by no means rare, especially amongst nurses in attendance on typhoid cases.* It is possible that infection may occasionally occur through the lungs, though this is unproven. Under certain conditions it seems probable that flies which have settled on typhoid excreta may convey infection, by mechanical means, to articles of food. The primary seat of infection is the intestine, and this probably remains the chief centre of the disease. But the bacilli not only infect the mesenteric lymphatic glands, but pass by the blood stream to distant organs. In this way the kidneys or bladder often become infected, so that the urine may contain the bacilli in enormous numbers. It has also been shown that they may be present in the sputum. These possible sources of infection have, therefore, to be taken into account, in addition to the primary risk from the stools. In order to prevent the spread of the disease all excretions and discharges from typhoid cases must be subjected to thorough disinfection. Where conveniences exist for cremating them, this is the safest method. If they have to be poured away down a water-closet, they should be intimately mixed with carbolic acid of such strength that the carbolic acid forms at

* Koch has recently (1903) brought forward evidence to show that the endemic typhoid in certain German villages is due to direct infection and not to polluted water.

least 1 part in 40 of the total mixture, all lumps being well broken up. They should stand for some hours in contact with the disinfectant before being poured away. Perchloride of mercury is not to be recommended, but chloride of lime (1 part to 500 of the total mixture) or izal (1 part in 200 of the total mixture) are both good disinfectants for typhoid stools. In the absence of a water-closet system, typhoid excreta should be mixed with chloride of lime and deeply buried, remote from any well, or other water supply liable to pollution. In addition to these precautions, which are designed to prevent widespread infection, there are others which are needful to prevent the direct infection of those in attendance on typhoid cases. The essence of these is to prevent the transference of infection by the nurse's hands to her mouth, and hence attention to the hands is the main thing. When one considers the frequency of diarrhœa in typhoid fever, and the commonness with which the bedclothes and linen are soiled by the discharges, it is obvious that nothing can prevent frequent infection of the nurse's hands, and sometimes of her attire. It follows that, after any attention to a typhoid case which involves possible contamination by any excretion or discharge from the patient, a thorough cleansing of the hands is needful, lest at a subsequent meal bacilli be conveyed to the mouth. When a nurse is in a hurry there is a great temptation to be content with a perfunctory dip in the basin of 1 in 1000 perchloride of mercury solution which, in hospitals, usually stands at the foot of the typhoid patient's bed. But no reliance should be placed on this. Nothing can replace a thorough scrubbing of

the hands with soap, hot water and a nail brush, with especial attention to the nails, after which a rinsing in the perchloride solution is a suitable final proceeding. Before eating, the nurse should always repeat this process. It may be added that any one who is in serious fear of typhoid infection may secure a certain degree of immunity, though not an absolute one, by submitting to the anti-typhoid inoculation devised by Wright.

FIG. 24.—Vibrios of Asiatic Cholera, stained by the silver method to show their flagella. (Drawn from a microphotograph: magnification, 1000 diameters.)

**Cholera.**—The infecting agent is here well known. It is the Spirillum (or Vibrio) choleræ Asiaticæ, otherwise known as "Koch's Comma Bacillus." This organism forms no spores, and is capable of destruction with particular ease by heat and chemical disinfectants. It has a great objection to acids, and will not grow in any medium having the most faintly acid reaction; it is killed by the gastric juice in the healthy stomach, from which it follows that in cholera epidemics it is worth while to pay great attention to maintaining the stomach in a healthy condition, by avoiding errors in diet, treating indigestion, or even taking medicinal doses of dilute mineral acids. The path of infection is almost invariably by the mouth, from the drinking of polluted water. The vibrio can flourish as a saprophyte in impure water supplies. The channel by which infection leaves the body is in the stools, but in view of the discoveries which have been made in typhoid fever it would be unsafe to assume no other possible source of danger. The stools must be rigidly disinfected by the

methods mentioned under the heading of typhoid fever, or, if preferred, by the addition of an equal volume of 10 per cent. mineral acid (sulphuric, nitric or hydrochloric). This latter proceeding should not be carried out in metal vessels. Linen soiled by choleraic discharges must be soaked in 1 in 20 carbolic, or some equally powerful solution for twenty-four hours, or else boiled or steam-disinfected. Cases are on record in which cholera has been conveyed to laundry employées by infected linen. Vomited matters must be treated on similar lines, and it will be on the safe side to assume the infective properties of all discharges and excreta. As in the case of typhoid fever, the security of attendants upon cholera cases rests on rigid attention to cleanliness, especially of the hands, and especially in relation to the taking of food after any contact with the patient. A method of anti-cholera vaccination has been devised by Haffkine (analogous to that afterwards employed by Wright against typhoid fever), which conveys, according to available statistics, a certain degree of immunity, though not an absolute one.

**Malaria and Yellow Fever** may be considered together because they appear to be transmitted in an identical way. In malaria the parasite is now well known: it is not a bacterium but an organism belonging to the lowest group of animals—the Protozoa. At least three varieties of the "Plasmodium malariæ" are known to exist, corresponding to different forms of malarial fever, but their life histories are similar. In each it presents two phases—an asexual phase which is passed in the human body, and a sexual phase passed in the body of certain gnats belonging to a genus named

"Anopheles." When one of these gnats sucks the blood of a malaria patient, the parasites develop in its body and give rise to spores which after ten days are present in the salivary glands of the gnat, and if now the gnat bites a healthy person, the spores are conveyed into his blood and he develops malaria. In the case of yellow fever the parasite has not yet been certainly discovered: we do not even know whether it is animal or vegetable in its nature. But the experiments of the American observers in Cuba have shown plainly that it is conveyed by the bites of gnats and not by direct infection. The gnat is a different one to that which conveys malaria: its name is Stegomyia, and it bites by day, whereas the Anopheles bites by night. There may be other ways in which malaria and yellow fever are conveyed to man, but so far the only proved way is by gnat-bites. It follows that in these two diseases ordinary measures of disinfection are of no avail. Their spread may, however, be checked in several ways. First, by preventing the access of gnats to those suffering from the diseases, for the bites of uninfected gnats are harmless. Secondly, by the slaughter of the gnats, and especially the abolition of their breeding-places. Thirdly, by the avoidance of gnat-bites on the part of the healthy. In the case of malaria there is a fourth measure, viz., the destruction of the human blood parasites by quinine.

A number of other infective diseases remain to be considered in the closing lesson.

# X

## A SHORT ACCOUNT OF SOME OF THE BACTERIA CONCERNED IN INFECTION—(*continued*)

**Plague.**—The infecting agent in this disease is well known. It is a bacillus called "Bacillus pestis." It forms no spores and presents no special resistance against heat or chemical disinfectants. Plague is a disease common to man and certain of the lower animals, notably rats. These animals suffer extensively in plague epidemics and probably have no insignificant share in their spread. The idea that fleas play a part in conveying the disease from the rat to man, though not impossible, is by no means well proved. In the presence of the disease all rats found dead or ill should be destroyed, the bodies being cremated. Plague attacks man in more than one way. In the commonest form the bacilli penetrate the skin by some crack or abrasion, and reaching the nearest lymphatic glands give rise to the characteristic "buboes." In another, and even more dangerous, though less common form, the bacilli gain access to the body by the respiratory tract, and set up an acute pneumonia. In either case they are liable to pass into the blood and set up a general plague-septicæmia. This general infection is not uncommon

shortly before death and all the secretions may then become infectious. The pus from suppurating buboes contains the bacilli. All discharges and excreta from plague cases require disinfection after the methods previous described. The sputum in particular must be rigidly sterilised, preferably with chloride of lime. The risk of direct infection on the part of those in attendance on ordinary bubonic plague cases is not so great as might be supposed. The bacilli appear unable to penetrate the unbroken skin, so that the mere soiling of the skin with plague-infected material is probably harmless. But the most careful attention must be paid to any cracks or abrasions lest they become infected. It is probable that infection can take place through an intact mucous surface, such as the conjunctiva, or nasal mucous membrane. The act of coughing, in a plague patient, has thus infected a nurse. Reasonable care and scrupulous cleanliness on the part of a physician or nurse are commonly sufficient to prevent infection. But the danger is great in the pneumonic and septicæmic forms of plague. It is probable that the method of preventive inoculation devised by Haffkine produces a certain degree of immunity, and those in daily contact with plague patients should avail themselves of such protection as it can confer.

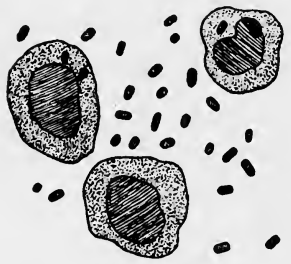

FIG. 25. — Plague bacilli, drawn from a preparation of the lung juice from a case of pneumonic plague. Three cells from the pulmonary exudation are seen, with numerous bacilli, stained more darkly at each end. (Magnification, 1000 diameters. Semi-diagrammatic.)

**Glanders** is fortunately a rare disease in man, but a very fatal, and an intensely infectious one. Its direct cause is the "Bacillus mallei," which is not known to form spores. It has about the same resistance to disinfecting agencies as the diphtheria bacillus, being destroyed fairly easily by heat and chemical disinfectants, but being able to withstand drying for some weeks. Remarkable care should be exercised in sterilising the discharges from a case of glanders, the greatest danger existing in those from the mouth and nose. In one form of the disease the skin is affected, and the bacilli are present in great numbers in the pustules. The risks run by those in attendance upon a case are by no means slight, and very scrupulous care is needed in disinfecting the hands after touching the patient or his soiled linen. Gargling, as described under diphtheria, is a useful preventive measure.

**Tuberculosis**, and, in particular, pulmonary phthisis, or consumption, is a disease in which it is now well recognised that disinfectant measures are demanded in the interests of the public health. The tubercle bacillus, upon infection with which the disease depends, is a slender bacillus which contains a good deal of fatty matter in its composition. It is not proved to form spores, though this point is not yet finally settled. It is possible that the fatty material it contains explains the fact that it is somewhat more resistant against heat and chemical agencies than most non-sporing bacteria. This resistance is less than that of true spores, but nevertheless it is advisable, in disinfecting tuberculous material, to rely only on such measures as will ensure the destruction of spores. The tubercle bacillus possesses a

very high degree of resistance against drying : the dried bacilli retain their virulence for many months. There can be little doubt that one of the most important ways in which tuberculosis spreads is by wholesale dissemination of the bacilli from the sputum of consumptives. In such sputum they may be present in incredible numbers; Nuttall calculated that a consumptive may spit up as many as three billion bacilli in twenty-four hours. It follows that disinfection of the sputum in consumption is a measure of the highest practical importance and that indiscriminate expectoration in public places is a crime against society. Every consumptive should carry a portable spittoon to receive his expectoration ; such vessels should be susceptible of easy sterilisation, or better still should be made of some cheap destructible material and burnt with their contents. Sputum, if not actually cremated, should be disinfected by boiling or by mixing with strong chloride of lime solution. What applies to sputum applies to all other tuberculous discharges, though the dangers from the sputum probably far outweigh the risk from other sources. But even if efficient disinfection of all tuberculous material from human cases could be secured, there would still remain another danger—that of infection from animal sources. Many of the lower animals, and especially domesticated cattle and pigs, are very prone to the disease. Recently, doubt has been cast by Koch on the identity of human and bovine tuberculosis, but his views have not been generally accepted. Until the matter is

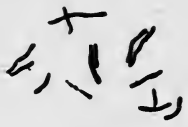

FIG. 26.—Tubercle Bacilli, drawn from a microphotograph. (Magnification, 1000 diameters.)

settled, it is certainly a wise thing not to underestimate the risk of infection from the milk and other products of tuberculous animals. It is a safe precaution to boil milk from unknown sources, at least for the consumption of children.

The risk of direct infection of those in attendance upon tuberculous cases is a very small one, provided that they are in good health and free from any inherited tendency to the disease. Tubercle is pre-eminently a disease which requires a susceptible soil for its development; most of us must frequently be exposed to infection, but the majority escape in virtue of the resistance of the healthy tissues. Nevertheless where infection is in a concentrated form, as in consumption hospitals, it makes its influence felt in a certain proportion of victims amongst nurses and others in daily contact with the sick. Free ventilation is a very important point.

**Leprosy** is due, in all probability, to a bacillus, closely allied to that of tubercle, which is found in abundance in leprous tissues, but has not yet been cultivated. The difficulty of communicating the disease to the healthy, even by direct inoculation, is so great that no fear of infection need be entertained by those in attendance upon the stray cases of leprosy seen in this country. It is probable that a very prolonged and intimate association with lepers is requisite to communicate the disease. Nevertheless all secretions from cases of the disease should be subjected to disinfection, and intimate contact with the patient as far as possible avoided. It is probable that measures adequate for the destruction of the tubercle bacillus will avail also against the bacillus of leprosy.

**Syphilis** is a disease, extremely communicable in its earlier stages, but only by direct contact, or by inoculation with the secretions in a tolerably direct manner. The infecting agent is not certainly known, but is possibly the bacillus described by Lustgarten, and having some affinities with the tubercle bacillus. It is so strict a parasite that it has never been cultivated. The primary syphilitic sore, or "chancre," furnishes a thin secretion which is intensely infectious. It is doubtful whether the unbroken skin can be infected: the chief danger lies in minute crevices or abrasions. It is probable that even an intact mucous membrane can be infected. In the secondary stage of the disease, when the general eruption appears, the secretions generally acquire highly infectious properties, and those of the mouth are an especial source of danger, more particularly in cases of syphilitic sore throat, or when mucous tubercles are present. In the eruptive stage the skin may also be a source of infection. The risk to others from a case of acquired syphilis in the secondary stage is present also during the active manifestations of congenital syphilis in infants. There is a general opinion, probably well founded, that in the tertiary stage of syphilis the danger of infection is much diminished, but it would be unsafe to assume that it does not exist. It follows from the preceding statements that a person who is the subject of syphilis is, during the primary and secondary stages at least, a source of grave risk to those brought into immediate contact with him. The disinfectant measures employed should be directed against a possibly spore-forming organism. Personal linen, bedclothes soiled by discharges, and everything

which can have become infected should be boiled or disinfected by steam. Special cups, spoons and other table utensils should be reserved for the sole use of the patient, and disinfected in boiling water. Dressings soiled by syphilitic secretions should be at once cremated. Where for any reason heat is inadmissible as a disinfectant, articles should be soaked for twenty-four hours in 1 in 1000 perchloride of mercury solution. A doctor or nurse whose hands have been in contact with secretions from a case of active syphilis should chiefly rely on thorough scrubbing with a nailbrush, soap and hot water, as in this way infective material is best removed from the crevices; the hands may afterwards be soaked in a disinfectant solution, such as 1 in 1000 perchloride of mercury.

**Soft Sore.**—In the soft, or non-infecting venereal sore, a small oval bacillus called, after its discoverer, Ducrey's bacillus, is present, and has some claim to be regarded as the true infecting agent. It appears to be a strict parasite and has not been cultivated on ordinary artificial media. There is no evidence that it forms spores, but no proof that it does not. The measures of disinfection required are those mentioned under syphilis.

**Gonorrhœa.**—The infecting agent in this disease is a well-known coccus, commonly known as the "gonococcus." It is a fairly strict parasite but can be cultivated outside the body, though only with difficulty and on special culture media. Infection is only by direct inoculation with gonorrhœal secretions, or by linen, etc., recently soiled with these. In the mucous membrane attacked, the gonococci insinuate themselves amongst the epithelial cells, which they may penetrate; they are

also contained in the pus-cells in large numbers. They may be scantily present in chronic cases of the disease for long periods, certainly for two years. It is wise to assume the infective character of a case of the disease so long as any discharge at all persists. In addition to its local presence in the affected mucous membrane, the gonococcus has been proved at times to pass to other regions of the body, *e.g.*, to the joints in gonorrhœal rheumatism, and to the peritoneum, where it may set up peritonitis. But for practical purposes of disinfection, attention should be concentrated on the affected mucous membrane. The gonococcus forms no spores, and its resistance to heat and chemical disinfectants is by no means great. Dressings contaminated by gonorrhœal discharges should be at once burnt, while linen and clothing soiled by them should be soaked in 1 in 20 carbolic solution, or 1 in 1000 perchloride of mercury For medical men and nurses who come into contact with cases of gonorrhœa, the great danger is conveyance of infection to the eyes, which may readily occur by means of the hands or by handkerchiefs if due care is not exercised. Should one eye become infected, the other should be protected by a watch glass and strapping. Strict attention to cleanliness is the most important detail in dealing with cases of gonorrhœa.

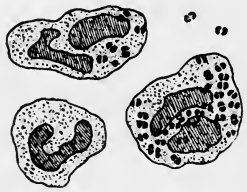

FIG. 27.—Gonococci, drawn from a stained preparation. Three pus cells are seen, two of them containing the paired cocci, with a few free cocci. (Magnification, 1000 diameters. Semi-diagrammatic.)

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There are a number of infections, falling for the most part within the province of the surgeon, which are

hardly to be classed with the specific fevers. They do not run a definite course, and are not contagious in the ordinary sense, requiring as a rule a definite wound infection. We may consider together such conditions as suppuration, cellulitis, erysipelas and septicæmia. Erysipelas and puerperal septicæmia have, it is true, some claim to rank with the specific fevers, but they may most conveniently be considered here. In addition to these, some remarks will be added on a few infective diseases such as anthrax, tetanus and rabies. It is necessary to consider such conditions as suppuration and septicæmia from two sides—as processes of disease, and also from the point of view of the micro-organisms which cause them. Suppuration may depend upon a variety of infections, and so may septicæmia; they are not specific diseases. The Streptococcus pyogenes may lead to cellulitis, abscess, or septicæmia; it causes no single specific disease.

**Inflammation** is a reaction of the tissues against an irritant—commonly, but not always, a bacterial irritant. It is, in a sense, a beneficial reaction, directed to the removal of the irritant. Hence one should not necessarily endeavour to check inflammation; treatment should rather be directed to the removal of its cause. *Suppuration* is an acute form of inflammation, in which the irritant produces a local death and softening of the tissues, and excites an excessive emigration of the white blood corpuscles, which form pus. Experimentally it may be produced by certain intense chemical irritants, but in surgical practice it is almost invariably due to bacterial infection. There are a large number of bacteria which may produce suppuration,

but there are some which are pre-eminently pus-producers, and they are hence known as "pyogenic organisms." In the first rank stand the pyogenic cocci—Staphylococcus pyogenes, Streptococcus pyogenes and Diplococcus pneumoniæ. The two first-named are the chief enemies of the surgeon. In addition to these, suppuration may sometimes be caused by Bacillus coli communis, Bacillus pyocyaneus and a few other bacilli, but far less commonly than by the pyogenic cocci. It may here again be noted that none of the ordinary pyogenic organisms form spores. The inflammation which results in abscess may be a purely local affair, as in an ordinary boil. The condition is more serious when it tends to spread along the lymphatics and to attack the lymphatic glands, or to extend far and wide amongst the connective tissues. These conditions are known respectively as *lymphangitis* and *cellulitis*, and they are almost invariably due to infection with Streptococcus pyogenes, which has a special habit of spreading along the lymphatics.

**Erysipelas,** in its classical form, is an acute spreading inflammation confined to the skin. It is due to infection by a Streptococcus which in all essential respects is indistinguishable from the Streptococcus pyogenes. Opinions are divided as to whether it is merely a variety of that organism, or a distinct but very closely allied species. Erysipelas is not uncommonly combined with cellulitis, and indeed the two conditions often pass into one another so that it is difficult to draw a hard and fast line between them. This fact is in favour of the view that their bacterial causes are varieties of the same streptococcus rather than distinct

species. If this view, which is held very widely, be correct, one would not necessarily have to search, in explaining the origin of a given case of erysipelas, for a pre-existing case of erysipelas from which infection must have arisen. Streptococcal infection from other sources might be enough to lead to it. It is true that erysipelas may spread from case to case in a surgical ward, and is a disease very properly isolated, but when single cases occur, it happens more often than not that no connection can be traced between them and any pre-existing case of true erysipelas.

**Septicæmia** is the term applied to the invasion of the blood by bacteria and their growth there. There are a large number of micro-organisms which can at times grow in the blood and spread far and wide throughout the body. But the great majority of cases of septicæmia are due to the pyogenic cocci—mostly to Streptococcus pyogenes. *Pyæmia* is a form of septicæmia associated with the formation of abscesses in the joints or internal organs; in this condition the infecting agent is more often the Staphylococcus pyogenes, though not necessarily so. *Puerperal fever* is merely a septicæmia in which the invading organism, generally the Streptococcus pyogenes—reaches the circulation from the uterus after child-birth. In septicæmia the bacteria commonly reach the blood by way of the small vessels, particularly the veins. If in a septic wound they enter the minute veins, they set up inflammation within them and cause a local clotting of the blood which tends to spread into the larger veins. This is called *septic phlebitis*, and the blood clot, which teems with the invading organisms, is very apt to become soft and crumbling,

particles being then carried away by the blood stream. But if a sufficient number of the bacteria get into the blood by any other channel, the result is the same.

It will be convenient to consider *seriatim* the individual species of bacteria which are concerned in the above and a few other "surgical" infections; many of them are as important to the physician as to the surgeon. Only a few brief statements can be made about each, chiefly from the point of view with which this book is concerned.

**Staphylococcus pyogenes.**—This is a coccus growing in clusters and not in long chains. There are two chief sorts, one forming an orange pigment in cultures and one being white. They are respectively called Staphylococcus pyogenes aureus, and Staphylococcus pyogenes albus, but their effects are practically identical and they are sometimes found growing side by side in the same abscess. The yellow variety is the more virulent, but they may here be considered together. Staphylococcus pyogenes, like other cocci, forms no spores, but, amongst non-sporing organisms, it is one of the most resistant known. It is killed in ten minutes by an exposure to a moist heat of 137° F. It can grow in broth cultures containing 1 part of carbolic acid in 500, but it is killed in a minute or two by an effective strength of 1 part of carbolic acid in 40 of the total mixture, or by izal, 1 in 200. It has however a peculiar resistance against perchloride of mercury; races of the coccus are sometimes found which can resist a solution of 1 in 1000 for half an hour or more. The salts of mercury should therefore be avoided in disinfectant measures directed against this particular organism.

It is a coccus commonly present as a saprophyte about the healthy body, both in the mouth and on mucous surfaces generally, and on the skin. One of the commonest skin bacteria is a white staphylococcus—the "Staphylococcus epidermidis albus" of Welch,—which seems only an attenuated and comparatively harmless form of it; but the Staphylococcus aureus also occurs on the skin. When virulent staphylococci gain access to the deeper tissues they are capable of setting up an extremely acute local inflammation, commonly ending in abscess. The majority of acute localised abscesses are due to this organism, but it fortunately has little tendency to spread along the lymphatics or to set up cellulitis. In deeper structures it is the commonest cause of acute suppurative affections of bone (osteomyelitis, acute periostitis). Should it by chance gain access to the blood and grow there, it sets up pyæmic conditions of the most dangerous kind, and it is the cause of the most rapidly fatal form of ulcerative endocarditis. Inasmuch as it is a normal inhabitant of the body surfaces, it is evidently liable to be a common source of wound infection, and its resistance renders it one of the surgeon's most serious enemies. It can be rubbed into the intact skin, entering the sweat glands or hair follicles, and thus arise boils on common seats of friction. In the treatment of boils it is of advantage to disinfect the skin for some distance around even when

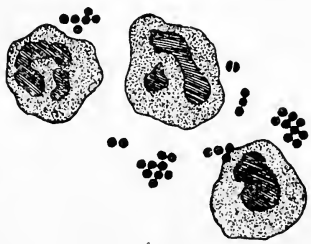

FIG. 28.—*Staphylococcus pyogenes aureus* in pus, drawn from a stained preparation. Three pus-cells are shown, with the cocci between them in irregular clusters. (Magnification, 1000 diameters. Semi-diagrammatic.)

they are healing, as in this way crops of secondary boils can often be averted. Carbolic acid or izal solutions may be used for this purpose.

**Streptococcus pyogenes.**—It is not certain how many sorts of streptococcus are able to cause disease in man. Their characters are very variable and it is difficult to distinguish between them. This difficulty has been already alluded to in speaking of erysipelas. The following remarks apply to the common Streptococcus pyogenes, which is by far the most important of the species causing disease in man. Streptococci are cocci growing in chains, and forming no true spores. They have less resistance than Staphylococcus pyogenes against heat and chemical disinfecants. Streptococcus pyogenes is killed by a moist heat of 130° F. in ten minutes. Carbolic acid (1 in 40) and izal (1 in 200) kill it in a minute or two, and it has not the peculiar resistance against perchloride of mercury which Staphylococcus pyogenes aureus exhibits. It is thus an organism tolerably easy to kill, but it can resist drying for some weeks or even a month or two. Streptococci are very abundant on the mucous surfaces of the healthy body and are especially numerous in the secretions of the mouth. Not all those species found in the mouth are capable of causing disease, but some of them can do so. Streptococci may also occur on the skin, but here they are less numerous than the Staphylococci. There is probably no one micro-organism which causes so many varieties of disease in man as does Streptococcus pyogenes and its near allies: and the diseases to which it gives rise are not only various, but very common and often very grave. They include "poisoned wounds,"

lymphangitis, cellulitis, and erysipelas, with suppurations, septicæmia, ulcerative endocarditis, and the gravest forms of inflammation in such serous cavities as the peritoneum or pleura. Infection of the skin is commonly through a scratch or wound, sometimes a very trivial one, but the size of the wound may bear no relation to the extent of the resulting inflammation, on account of the great power of spreading along the lymphatic channels which the Streptococcus possesses. The extent of the mischief depends in part upon the resistance of the tissues of the infected person, which varies in different persons and according to the state of health. But it also depends upon the degree of virulence possessed by the invading streptococci, and this also varies much. It is a fact, well established by animal experiment, that streptococci become greatly exalted in virulence when they have grown in the peritoneal cavity, and this is true also of the human peritoneum. There are no more venomous streptococci than those from a case of septic peritonitis, as many a surgeon and pathologist knows to his cost. The majority of serious post-mortem room infections arise from such cases, and they are not rarely fatal. It therefore behoves those who have to do with septic peritonitis (both while the patient is alive and after death) to take the most scrupulous care to avoid infection of chance scratches or abrasions.

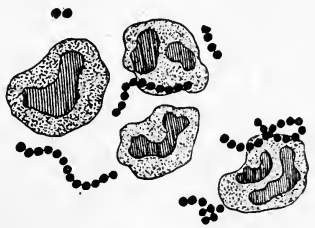

FIG. 29.—*Streptococcus pyogenes* in pus, drawn from a stained preparation. The cocci are arranged in chains, lying amongst the pus cells. (Magnification, 1000 diameters Semi-diagrammatic.)

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**Diplococcus pneumoniæ** (Pneumococcus).—This organism belongs in reality to the streptococci, and grows in chains in liquid artificial media. When growing in the animal body it is found usually as a paired coccus surrounded by a gelatinous capsule (*see* Fig. 22). It is a stricter parasite than Streptococcus pyogenes, and its life on artificial media is not a long one. It is commonly, but not always, present in the mouth secretions in health, and this is all we know of its habitat apart from diseased conditions. It forms no spores, and is killed by heat and chemicals rather more easily than is Streptococcus pyogenes. It does not readily attack the healthy tissues in man: their resistance must be lowered by chill or other predisposing cause of disease. It is therefore not an organism against which any energetic measures of disinfection are demanded. Its relation to pneumonia has been already mentioned. Its interest to the surgeon rests in the fact that it may be a cause of suppuration in certain regions of the body. It is the commonest infecting agent in empyema, and it may cause middle-ear disease, suppurative meningitis, and, more rarely, peritonitis.

**Bacillus coli communis** (or Bacterium coli commune). This is a motile bacillus, forming no spores. While it is killed with tolerable ease by heat and chemical disinfectants, its resistance against these is somewhat higher than that of many non-sporing organisms, though not so great as that of Staphylococcus pyogenes. It owes its importance in medicine and surgery to the fact of its extreme abundance in the intestinal contents, especially in the large intestine, so that the smallest particle of fæcal material is capable

of conveying infection to a susceptible soil. In the intestine it lives as a saprophyte, and it is capable of growing in this way outside the body with much vigour and hardiness. It does not readily attack healthy tissues, but it can attack those weakened by disease or injury. It is thus liable to spread from the intestine and assume the *rôle* of a parasite under a variety of conditions. Should fæcal material gain access to the peritoneal cavity from perforation of an intestinal ulcer, or in the course of a surgical operation, the resultant peritonitis is very commonly associated with the growth of this organism. In chronic inflammation of any part of the intestine, for example, of the vermiform appendix, Bacillus coli communis may make its way through the weakened tissues to the peritoneal coat, and set up a localised peritonitis. The inflammation set up by this bacillus is commonly not of great intensity, but at times it passes on to suppuration; thus abscesses may arise in connection with different parts of the intestinal tract. The mucous membrane of the urinary bladder is not rarely infected by B. coli communis, which is a common cause of cystitis, and from the bladder infection may ascend to the kidneys. Where acute spreading gangrene follows upon a severe lacerated wound this bacillus has sometimes been found in the dying tissues, apparently playing some part in the gangrenous process, though usually associated with anaërobic bacilli. Bacillus coli communis varies much in virulence. When derived from the healthy intestine it is usually far more harmless than when derived from the diseased intestine or from some secondary focus of infection. There are varieties, or perhaps closely allied

species, which seem capable of setting up epidemics of infectious disease in animals and occasionally in man. The infectious form of septic pneumonia already alluded to is a case in point. Apart from such clearly infectious conditions, there appears no need to employ disinfectant measures against B. coli communis so far as the risk of infecting others is concerned; but there is evidently very great need, in surgical operations involving any part of the intestinal tract or any region inhabited by this bacillus, to use the most rigid precautions against the infection of the sound tissues of the patient by fæcal matter or infected material generally.

**Bacillus pyocyaneus** (Bacillus of blue pus).—This is a motile bacillus which produces a bright blue pigment. It forms no spores, and is destroyed readily enough by the ordinary means effective against non-sporers. It is capable of causing septicæmia and abscess in animals, but has very small importance in human disease. Wounds accidentally infected with it secrete bluish or greenish pus, but their healing is not much interfered with. The mode of infection is uncertain: the bacillus has been found in the air.

**Actinomyces** (Ray fungus).—This organism, or rather group of organisms, is not a true bacterium, but belongs to the genus Streptothrix, intermediate between the bacteria and the higher fungi. The ray fungus consists of a feltwork of fine branching threads, the extremities of which, at the edge of the mass, are usually swollen in a clubbed fashion. When grown on artificial media rows of spore-like bodies are formed in the branches which project into the air, but these do

not appear to correspond to the endospores of bacteria and have far less resistance against heat. The most resistant forms are destroyed at 167° F. in less than half an hour, and many are killed at a much lower temperature than this. The resistance against chemical disinfectants is imperfectly determined: it is probable that it is somewhat greater than that of non-sporing bacteria, but less than that of true spores. The forms of Streptothrix which affect man cause a chronic suppuration, which slowly spreads, and may give rise to a pyæmic condition. Infection is commonly by the mouth, and is believed to take place through cereals, especially barley, upon which the fungus naturally grows.

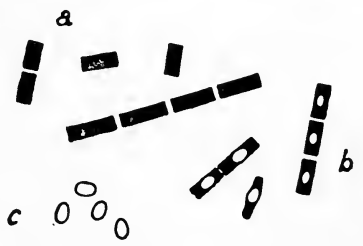


FIG. 30.—Anthrax bacilli. At (*a*) are seen the non-sporing vegetative forms: at (*b*) the stages of spore formation, and at (*c*) a group of free spores. (Magnification, 1000 diameters. Diagrammatic.)

**Anthrax** (Malignant pustule; woolsorters' disease).—The anthrax bacillus is a non-motile, stout, square-ended rod, growing in long chains. It is a typical example of a spore-forming bacterium, but it forms its spores only in contact with air and not within the animal body. The spores are killed by boiling (212° F.), usually in two minutes, but they may require ten minutes. Dry heat requires an even more prolonged exposure and a higher temperature —284° F. for several hours. They can withstand drying almost indefinitely. Perchloride of mercury solution (1 in 1000) kills them in two to three hours, but 1 in 20 carbolic solution may take many days to do so. The non-sporing bacilli, on the other hand, are

killed with comparative ease, having less resistance than Staphylococcus pyogenes. Man is hardly ever infected from human cases of the disease, though the danger exists. Anthrax is essentially a disease of animals, and occurs especially in cattle, sheep, and horses. The vast majority of cases of human infection arise from hides, wool, and hair imported into this country in an infected condition (*i.e.*, containing anthrax spores). No satisfactory method of disinfecting such infected material without spoiling it has yet been devised. The common form of human infection is by the skin, a slight abrasion becoming infected by the spores. The result is a local inflammation, which may, if untreated, lead to a fatal result from general infection. Infection is sometimes by the lungs, from inhalation of spores (woolsorters' disease), and this is invariably fatal.

**Tetanus.**—This disease is due to a bacillus, slender, richly provided with flagella, strictly anaërobic, and forming true spores. The spores are formed at one end of the rod, giving it the appearance of a drumstick. The resistance of these spores to heat and chemical disinfectants is fully equal to that exhibited by anthrax spores—if anything, slightly greater. The tetanus bacillus lives in earth, especially in manured garden soil and in street mud. That infection is not common in gardeners and those whose scratched hands come much into contact with earth presumably depends on the strictly anaërobic character of the bacillus. Infection of a superficial wound seems to produce no result; to cause tetanus the bacilli must be deeply implanted, as happens in deep lacerated and punctured

wounds contaminated with dirt or mud at the time of their production. The bacilli, when thus implanted in the damaged tissues away from the free oxygen of the air, are able to grow. They remain localised in the wound, usually in small numbers, but the poison they produce is a very intense one, and being absorbed, causes the severe and often fatal spasms of tetanus. Disinfection of the discharges from tetanus wounds is thus of little moment to the community at large, or even to those directly attendant upon the patient; but the cleansing of lacerated or punctured wounds from earth and mud as soon as possible after their infliction is of very great moment. Mechanical cleansing is the most important detail, for it is impossible to employ chemical disinfectants strong enough to kill tetanus spores. In the after-treatment of a wound which has been contaminated with material liable to contain tetanus spores free access of air to the tissues is to be recommended, and it may be well worth while to inject a prophylactic dose of anti-tetanus serum. Horses and other animals can easily be immunised against tetanus by the ordinary methods, and the serum of such immunised animals is capable of conferring protection upon other animals. In practice this serum has not fulfilled all the hopes to which, on its introduction, it gave rise. It has little curative effect when once the symptoms of the disease manifest themselves; but its prophylactic value is well established, and its

FIG. 31. — Tetanus bacilli, drawn from a stained preparation of a pure culture. The flagella are not represented. The various stages of sporulation are depicted, the spores appearing as bright spaces at one end of the rods. (Magnification, 1000 diameters.)

highest value lies probably in cases in which tetanus is to be feared but has not yet become apparent; in other words, the antitoxin, if it is to do much good, must have the start of the toxin.

**Rabies** (Hydrophobia).—In this disease the exact nature of the infecting agent is still unknown. But it is well established that in a rabid animal the poison is chiefly concentrated in the central nervous system, and is also present in the saliva; this is doubtless also true of human cases. The poison is easily destroyed by heat, *e.g.*, by exposure for an hour to 122° F., and it gradually loses its virulence on drying. It cannot, therefore, well be due to a spore-bearing organism. As in the case of tetanus, the poison must be deeply implanted in the tissues in order to produce the disease. There is little risk of infection from human cases of rabies: the disease is practically always conveyed by the bites of rabid animals. As there is every reason to suppose that the causal agent is a strict parasite and cannot grow apart from the animal body, it follows that if the disease can be exterminated in animals it will cease to occur in man. This is confirmed by the effect of the muzzling orders in this country. In the treatment of wounds inflicted by rabid animals, speedy cleansing and disinfection is essential. After a preliminary washing, the actual cautery should be freely applied. The patient should in all cases be sent to a Pasteur Institute for treatment.

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In conclusion, it may be of service to give a table of the principal infective diseases attacking man, classified according to what is known as to their

power of forming spores, as some guide to practical disinfection.

1. *Diseases in which the infecting agent is well known* and in which there is a *reasonable certainty that it forms no spores.*

Typhoid fever (enteric fever).
Malta fever (Mediterranean fever).
Asiatic cholera.
Plague (bubonic plague).
Diphtheria.
Glanders (farcy).
Epidemic cerebro-spinal meningitis.
Erysipelas and cellulitis.
Septicæmia and pyæmia (in their common forms).
Infective endocarditis (ulcerative or malignant endocarditis).
Suppurations—abscess—boils, &c. (in their common forms).
Gonorrhœa.
Acute pneumonia.

To these may be added the following, in which the resistance of the infecting agent is a little higher than in ordinary bacteria, although there is no proof that true spores are formed:

Actinomycosis.
Tubercle.
Leprosy (in all probability).

2. *Diseases in which the infecting agent is less certainly known*, or even unknown, but in which there is a *presumption that it forms no spores*

Typhus fever.
Relapsing fever.
Scarlet fever (scarlatina).
Measles.
Rubeola (German measles, rötheln).
Influenza.
Whooping cough.
Mumps (epidemic parotitis).
Rheumatic fever (acute rheumatism).
Dysentery.
Syphilis.
Rabies (hydrophobia).

3. *Diseases in which the infecting agent is imperfectly known or unknown*, but in which there is *some ground for supposing that it may form spores.*

Small pox (variola).
Vaccinia (cow-pox).
Chicken pox.
Diarrhœa (some acute forms).

4. *Diseases in which the infecting agent is well known and certainly forms true spores.*

Anthrax (malignant pustule, woolsorters' disease).
Tetanus (lockjaw).
Malignant œdema (acute spreading traumatic gangrene).

In addition to these groups there are the two diseases —malaria and yellow fever—in which infection, so far as is yet proved, is conveyed only by insect bites.

There is also the group of parasitic skin diseases, such as ringworm, favus, etc., which are due to well-known fungi, forming spores like other moulds. The exact resistance of these spores to disinfectant agencies is very imperfectly known, but it is probable that it lies between that of non-sporing bacteria and that of true bacterial spores.

# PART II

## PRACTICAL EXERCISES AND DEMONSTRATIONS illustrating the teaching of the foregoing lessons

**Introduction.**—It has been already remarked that a short course of practical work in the laboratory will do far more in teaching the principles of disinfection than the reading of many books. Real living knowledge can only come from actual experience. Such experience may be gained by an easy series of experiments such as will here be suggested. It is by no means necessary that the learner should undertake a complete course of practical bacteriology. It is hardly too much to say that all the essential truths concerning disinfection and sterilisation can be illustrated without the use of a microscope, by a course of what may be termed *naked eye bacteriology*. The majority of the experiments here mentioned are, in fact, of this naked eye character. It is nevertheless of advantage, when possible, that the learner should acquire the power of using a microscope in the examination of bacteria, and this is included in the scheme of practical work. The technique of staining bacteria is, however, altogether excluded as unessential to the immediate object of the work. The making of cultures, on the other hand, is of much importance,

and it has also been deemed of advantage to include in the practical work the making of a simple culture medium, on account of the valuable training in the principles of sterilisation which it affords.

In order to carry out even the simplest course in practical bacteriology, access to a properly equipped laboratory is essential, and in the following experiments the supervision of a competent practical teacher is also taken for granted. Much of the minute detail upon which the success of the experiments depends is left undescribed in the text, as it can only properly be learned from the practical demonstrations of the teacher. Indeed, the whole course of practical instruction may be varied at will according to his discretion. Some of it must necessarily be of the nature of demonstration rather than true practical work. The course here mapped out is thus largely a series of suggestions to the teacher, based upon the writer's own experience in teaching the subjects dealt with. The experiments have been verified with great care in the laboratory, and often many times repeated to ensure their accuracy. As far as possible the practical work is described in the order in which the subject-matter has been treated in the preceding lessons, but as many of the exercises require several days for their completion, the order of the experiments and demonstrations must depend upon the judgment of the teacher and the time at the disposal of the class. The metric system of weights and measures, which alone is used in laboratory work, is here adhered to, although in the text of the lessons it has been avoided as less familiar to the popular mind.

The first four lessons and, in part, the last two, must

be practically illustrated by demonstrations rather than by experiments. The time devoted to practical work may at first be usefully employed in learning the use of the microscope, the art of making cultivations, and in the making and sterilising of a culture medium.

**The Use of the Microscope** for bacteriological study is a valuable adjunct to the course of practical work, but is not absolutely essential. The learner will require to be taught the uses of the different parts of the instrument, and in particular of the substage condenser, the diaphragm, and the plane and concave mirrors. The magnifying powers of the different objectives and eye-pieces must be explained and illustrated on a hair or filament of cotton wool. The mode of using oil-immersion lenses must be shown, and the principles of focusing with the coarse and fine adjustments made plain.

Unstained, living preparations of various harmless bacteria, yeasts and moulds, may then be demonstrated. The motility of certain bacteria can be shown, and Brownian movement distinguished from it. The learner may then be encouraged to prepare and examine for himself similar specimens from cultures provided, or from the colonies which have arisen on a plate cultivation which has been exposed to the air, for five minutes, four or five days previously. This will involve teaching the mode of using the mounted platinum wire, and sterilising it by heat after use. Care must be taken to emphasise the necessity of using a narrow diaphragm and concave mirror in examining unstained preparations. Stained films of micro-organisms may then be demonstrated under a $\frac{1}{12}$-inch oil-immersion lens, with

plane mirror and open diaphragm, but the preparation of such stained specimens is not an essential part of the course.

In the above ways the student may soon learn to recognise the different forms of micro-organisms described in the first lesson, and to tell the difference between a staphylococcus, a streptococcus and a sarcina, between a motile and non-motile bacillus, and to distinguish between moulds, yeasts, bacteria and streptothrices. More than this is not requisite for the present purpose.

The following demonstrations are suggested to illustrate the facts stated in the second and third lessons:

The *rate of multiplication of bacteria* may be illustrated in hanging-drop cultivations of some rapidly growing organism, such as Staphylococcus pyogenes. A freshly made culture should be shown side by side with one which has been incubated for twenty-four hours at 37° C. In the latter the process of fission can be seen in its various stages. Or the same thing may be made plain to the naked eye by exhibiting (*a*) a broth tube just inoculated with the staphylococcus, showing no turbidity, and (*b*) a similar tube incubated for twenty-four hours at 37° C. The number of organisms present in a single drop of the latter may be demonstrated under the microscope.

*Spore formation* may be demonstrated by fresh and stained preparations from cultures of Bacillis subtilis of different ages (from eighteen to forty-eight hours).

The *vegetable nature* of bacteria may be proved by exhibiting Bacillus pyocyaneus, or some other hardy saprophyte growing in Pasteur's fluid. (This consists

of ten parts of cane sugar, one part of tartrate of ammonia, and the ash of one part of yeast dissolved in 100 parts of water.)

*Aërobic and anaërobic* modes of growth. These are most readily illustrated by gelatin stab-cultures prepared four or five days beforehand. Staphylococcus pyogenes and Bacillus anthracis are convenient aërobes; the tetanus bacillus, or the bacillus of malignant œdema, convenient anaërobes. Of the latter, deep glucose-gelatin stab-cultures must have been prepared.*

The varying *temperature requirements* of bacteria. Two similar cultures should previously have been prepared of each of the following: (*a*) the pneumococcus or the tubercle bacillus and (*b*) B. filamentosus, or some other water organism which will not grow at body temperature. One of each pair is to be incubated at 20° C. and one at 37° C.

The effects of *light* may be shown by a gelatin plate culture of the cholera vibrio, upon the bottom of which has been stuck a broad black paper cross, and which has been exposed for several days, bottom upwards, to diffuse daylight. The cross is then to be removed to show the more vigorous growth which has taken place in the protected parts.

The *distribution* of bacteria in nature may be illustrated by cultivations previously made from the skin and mouth, from water and sewage, and by exposing plates

* In the case of all cultures required for demonstration, much trouble will be saved by preparing and fixing a permanent set. This is done by exposing the cultures, when sufficiently advanced in growth, to strong formalin vapour for a few weeks. The tubes may then be sealed with the blowpipe. Such specimens remain good for many years.

to air. The actual numbers of organisms present in water, saliva, sewage, etc., may be shown by plate cultures made with measured quantities of the fluids (in the case of saliva, use 1 cc. of a million-fold dilution for an agar-agar plate, to be incubated at 37° C.; in the case of sewage, 1 cc. of a hundred-thousand-fold dilution will suffice).

**Putrefaction.**—The following experiment may be readily performed by the learner himself under supervision. Chop up a little meat, raw or cooked, and put a pinch of it into each of three sterile broth tubes. Replace the cotton wool plugs, and steam the three tubes for half an hour. Inoculate one of the tubes with Proteus vulgaris or Proteus Zenkeri and incubate it, aërobically, at 37° C. To another, add a small pinch of garden earth (which always contains an abundance of the spores of anaërobes), and incubate this at 37° C., but anaërobically, in a Buchner's tube, or whatever other form of apparatus for growing anaërobes is present in the laboratory. The third tube is for a control; incubate it aërobically with the first tube without inoculating it with anything further. After two or three days' incubation examine the three tubes and compare them as to appearance, smell, etc. The control tube may or may not be sterile (a few spores may have escaped the single steaming), but it will be quite sweet. The aërobic tube inoculated with Proteus will be turbid and will have a faint smell, but not a very offensive one. The anaërobic tube will be extremely offensive. Examine the fluid in the aërobic and anaërobic tubes microscopically and compare the characters of the bacilli present. The experiment may be made more complete by pre-

paring sub-cultures from the two tubes, as follows. Melt two culture tubes, one of plain gelatin and the other of glucose gelatin. Inoculate the former with the minutest possible trace of the aërobic cultivation and gently shake it. Inoculate the latter with a somewhat larger amount of the anaërobic cultivation, and heat it to 80° C. for ten minutes to kill everything except spores. Let both tubes set, and incubate them side by side at 20° C. for three or four days. The Proteus colonies from the aërobic culture will be distributed all through the gelatin and will be growing best at the surface. The anaërobic colonies from the other culture will have grown away from the surface only (but if aërobic spores chanced also to be present these may have developed at the surface).

*Fermentations.*—Alcoholic fermentation by yeast may be demonstrated in the usual way in glucose broth, the gas formed being collected in a small test-tube introduced, inverted, into the culture medium before sterilisation. The souring of milk may be demonstrated by sterile milk tubes inoculated with the lactic acid bacillus.

The *saprophytic bacteria* present in the mouth (or intestine) and on the skin will already have been demonstrated by cultivation. *Parasitic bacteria* may be shown in stained sections of various affected tissues, *e.g.*, of an anthrax pustule, of tuberculous lung, etc. Cultures also from diseased tissues may be shown. *Local and general infection* may be respectively illustrated by sections of diphtheritic membrane "in situ," and by cultures from the blood of a case of septicæmia.

Practical work illustrating the lesson on the **cultiva-**

**tion of bacteria** is of such importance that considerable attention should be devoted to it from the first, because the making of cultures forms an integral part of the experiments on disinfection which are to follow, and which it is most desirable that the pupil should carry out in person. As much practice as possible should be given in the manipulations required for making the different sorts of cultures, harmless species being, of course, used for the purpose. This may be done, at the teacher's discretion, when time allows during the hours devoted to practical work after the first four lessons. The different forms of natural and artificial culture media in use will thus become familiar. The principles which govern the making of cultivations have been indicated in the text of the fourth lesson. The actual details must be learned under the direct supervision of the teacher. The pupil must be shown how to make streak-cultures, stab-cultures, shake-cultures and plate-cultures, and carefully watched at all stages. A number of cultures will be required for the practical work of the lessons to follow, and these may be prepared by the pupil. Suitable bacteria are Staphylococcus pyogenes aureus, Bacillus coli communis and Bacillus subtilis.

If it is possible to arrange the time for it, a most valuable practical exercise at this period will be the actual making and sterilising, through all its stages, of a simple culture medium such as peptone-broth. Since its preparation extends over several days special times of attendance in the laboratory must be arranged. The details of the process are not given here, since they are a matter of common routine in every laboratory. The value of the exercise lies in the practical training in

sterilisation, and the evident need for careful attention to detail at every stage. Further, the success or failure of the means employed is readily apparent, if a few of the finished broth tubes are incubated for forty-eight hours at 37° C.

## DISINFECTION BY HEAT

The following simple experiments illustrate the chief facts of heat disinfection.

**Thermal death-points of non-sporing bacteria.**—Staphylococcus pyogenes aureus is a good species to test on account of its high resistance. Bacillus coli communis may be compared with it. Broth cultures of these organisms, or of any others it is desired to test, should have been made the day beforehand. A hot water bath, which with a little attention can be kept at a constant temperature, must be prepared; the best form consists of an iron or copper cylinder holding three or four pints, heated by a Bunsen burner from below, and with a lid perforated by holes which just fit the test-tubes. A thermometer passes through the lid into the water. A number of sterile broth tubes or sloped agar-agar tubes must be in readiness, and it is convenient to label these beforehand in accordance with the details which follow. When all is ready heat the water bath, which should be nearly full of water, to exactly 60° C. The learner should feel the bath with the hand to realise what this temperature means. Now insert the broth culture tubes of the organisms to be tested through the holes in the lid so that the level of the broth is completely beneath the surface of the

water. Note the exact time by a watch. Sub-cultures are to be made by transferring a platinum loopful to one of the sterile tubes in readiness, at stated intervals, say every two minutes. This must be continued for twenty minutes, *i.e.*, ten sub-cultures in succession from each broth culture tested. The work can be divided: one pupil can keep time by the watch, another can watch the thermometer, applying fresh heat from below so as to keep it constant at 60° C., while others make the sub-cultures. When all the cultures are made, incubate them for forty-eight hours at 37° C. and then examine them. In the case of B. coli communis it will probably be found that death has taken place in from six to eight minutes from the commencement of the experiment. In the case of Staphylococcus aureus life will be found to have been maintained for some minutes longer, to ten or even twelve or fourteen minutes (various strains differing in their precise length of resistance). The later sub-cultures should, in each case, be sterile. To complete the experiment make an agar-agar sub-culture from the latest tube which has grown, in order to be sure that the growth is not an accidental contamination, but truly the organism tested.

**Thermal death-point of spores.**—For testing this the very resistant spores of the hay bacillus (B. subtilis) are suitable. With due supervision on the part of the teacher the less resistant spores of the anthrax bacillus may be compared with them. Sloped agar-agar cultures of one or both of these organisms should have been inoculated three or four days beforehand and incubated at 37° C. Having examined the cultures microscopically to be sure that they contain an

abundance of fully formed, free spores, make an emulsion of the growth in 5 cc. of sterile water in a test-tube, using two or three loopfuls so that the emulsion is quite turbid. Put a beaker of water on a sheet of wire gauze supported by a tripod, and bring it up to a brisk boil over a Bunsen burner. Have in readiness a number of sloped agar-agar tubes, or broth tubes, ready labelled for the experiment. Now bring the emulsion of spores rapidly up to boiling-point over the naked flame, and note the exact time at which it begins to boil. At this moment plunge it into the beaker of boiling water, the water level in which should be above that in the test-tube, so that the fluid in the latter is quite surrounded by boiling water. Sub-cultures on agar-agar, or in broth, are to be made every few minutes, up to half an hour. Should the water in the beaker boil away to too low a level fresh *boiling* water must be added to make good the loss. It is a good plan to place also in the beaker another test-tube containing plain water. The exact temperature in this can be taken with a thermometer and compared with that of the water in the beaker. It will be found to be about 1° C. lower than the latter—*i.e.*, the spores are being exposed to a temperature about a degree short of the boiling-point. The sub-cultures are to be incubated at 37° C. for two days. The spores of the hay bacillus will probably be found to have survived for fifteen to twenty minutes—those of the anthrax bacillus for a much shorter period. The period of survival may be a little shortened by substituting for the water in the beaker a strong solution of washing soda. This boils at a slightly higher temperature than plain water, and so the spores in the test-tube

can fairly be exposed to 100° C. Some fragments of broken glass should, in this case, be put in the beaker to check "bumping."

This method of testing the thermal death-point of spores may be supplemented by the following experiment: Prepare an emulsion of the spores in a test-tube, as above, and then boil it continuously over the naked flame of a Bunsen burner instead of immersing it in boiling water. Make sub-cultures at intervals of a minute from the moment when boiling commences. It will be found that even hay bacillus spores are killed in one or two minutes by this process. (A thermometer in the boiling fluid will only register 100° to 102°., and the difference in the result is probably to be explained by the much higher temperature of the glass tube exposed to the naked flame, and with which the spores come into contact.)

**Steam Disinfection.**—If a good steam disinfecting apparatus be accessible for the experiment, the following test will be none too severe. (Failing a proper steam disinfector, a similar experiment on a smaller scale can be devised and carried out in an autoclave.)

Take an old Directory or other thick book, of no value—the bigger and thicker the better. Open it in the middle and with a pen-knife cut a small cell in the central pages. In this cell place some fragments of paper smeared with a sporing culture of the hay bacillus. No aseptic precautions need be used at this stage. Close the book, and make it up into a parcel with brown paper and string. Wrap it up in old cloths, blankets, or other articles till the parcel is of considerable bulk, and again finish with brown paper and string. Then send

it to the steam disinfector to be passed through with other goods. Afterwards remove the wrappings. Have ready some broth tubes. Open the book cautiously and with sterilised forceps transfer the spore-infected fragments of paper to the broth tubes—avoiding all unnecessary exposure to the air. Incubate the cultures at 37° C. They should remain perfectly sterile, indicating absolute sterilisation to the very centre of the book. Note the condition of the book: the binding will be distorted and spoiled, but the paper uninjured. The book, if rebound, would be as good as ever.

## CHEMICAL DISINFECTION

### Testing the restraining and germicidal Powers of Chemicals.

(1) *The restraining Effect of Carbolic Acid.* Staphylococcus pyogenes aureus and B. coli communis are suitable organisms for this experiment, which may be carried out on each separately and the results compared.

A number of sterile broth tubes must be in readiness, each containing just 10 cc. of broth. Prepare carbolic acid solutions of the strength of 1 in 20 and 1 in 40. With a long 1 cc. pipette, graduated in tenths, add to different broth tubes the following amounts of the disinfectant:

| | | | | | |
|---|---|---|---|---|---|
| (1) | 2.5 cc. | of 1 in 20 | carbolic acid | to 10 cc. broth | = 1 in 100 |
| (2) | 2.5 cc. | „ 40 | „ | „ | = 1 in 200 |
| (3) | .8 cc. | „ 20 | „ | „ | = 1 in 270 |
| (4) | .7 cc. | „ „ | „ | „ | = 1 in 306 |
| (5) | .6 cc. | „ „ | „ | „ | = 1 in 353 |
| (6) | .5 cc. | „ „ | „ | „ | = 1 in 420 |
| (7) | .4 cc. | „ „ | „ | „ | = 1 in 520 |
| (8) | .25 cc. | „ „ | „ | „ | = 1 in 820 |

Now inoculate each tube with a liberal trace from a young agar-agar culture of Staphylococcus pyogenes aureus, and incubate the tubes at 37° C. for two days. On examination it will be found that No. 8 has probably grown well; even No. 7 may show good growth. In Nos. 5 and 6 growth, if it has occurred at all, will be very scanty. From No. 4 upwards no growth is likely to occur. From the last tube (*i.e.*, that which contained most carbolic acid) which has grown at all, make a sub-culture on a sloped agar-agar tube, to prove that the growth is truly Staphylococcus aureus. At the same time make sub-cultures on agar-agar from the tubes which show no growth. This is best done by taking up some of the deposit from the bottom of the tube with a freshly made capillary pipette. Incubate these agar sub-cultures for two days at 37° C. It will be found that No. 4 and possibly No. 3 were still living after forty-eight hours in the carbolic acid, but the sub-cultures from Nos. 1 and 2 will be sterile. It will thus be proved that carbolic acid in a strength of 1 in 200 ($\frac{1}{2}$ per cent.) kills Staphylococcus pyogenes aureus in forty-eight hours. In a strength of 1 in 300 ($\frac{1}{3}$ per cent.) it restrains growth, but does not actually kill in this time. In a strength of 1 in 500 ($\frac{1}{5}$ per cent.) it does not prevent growth. A similar experiment should have been carried out at the same time with B. coli communis, and the results compared.

(2) *The restraining Effect of Perchloride of Mercury upon Bacillus subtilis Spores.*—As in the preceding experiment, have ready a number of sterile broth tubes containing exactly 10 cc. Make up accurate solutions of perchloride of mercury in distilled water,

of the strengths 1 in 1000 and 1 in 2000. With a graduated 1 cc. pipette add to the different broth tubes the following amounts of the disinfectant:

| | | | | |
|---|---|---|---|---|
| (1) .8 cc. of | 1 in 1000 | perchloride | = 1 in | 13,500 |
| (2) .4 cc. | „ | „ | = 1 in | 26,000 |
| (3) .3 cc. | „ | „ | = 1 in | 34,333 |
| (4) .2 cc. | „ | „ | = 1 in | 51,000 |
| (5) .1 cc. | „ | „ | = 1 in | 101,000 |
| (6) .1 cc. | „ 2000 | „ | = 1 in | 202,000 |

Now inoculate each tube from a sporing culture of B. subtilis, and incubate them for forty-eight hours at 37° C. The probable results will be as follows: No. 6 will show fairly good growth, as a pellicle on the surface; No. 5 will show slight general turbidity, but probably no surface pellicle; No. 4 may appear sterile, but delayed growth may appear in this, too, after three or four days. The other three tubes should show no growth at all, even on prolonged incubation. Yet an agar-agar sub-culture from No. 1, made with a capillary pipette from the deposit at the bottom, will grow well, showing that in a strength of 1 in 13,000 the perchloride of mercury has merely acted as an antiseptic, inhibiting growth but not killing the spores.

(3) *Testing the germicidal Powers of Carbolic Acid upon non-sporing Bacteria.*—Suitable organisms are Staphylococcus pyogenes aureus and B. coli communis. Broth cultures of these should have been prepared a day previously, or, if preferred, agar-agar cultures, from which emulsions may be made in broth or sterile water. The emulsions should be free from lumps, which may be attained by filtering them through sterilised filter paper. Make up an accurate solution of carbolic acid

of the strength of 1 in 20. Have in readiness an empty sterile test-tube and a couple of sterilised 5 cc. glass pipettes. Measure out with one of the pipettes 5 cc. of the bacterial culture or emulsion into the empty test-tube, and at a given moment, timed accurately by a watch, add 5 cc. of the 1 in 20 carbolic solution and mix rapidly; the total strength of the mixture will be 1 in 40 carbolic. The moment the mixture is made, begin to make sub-cultures by transferring a platinum loopful to successive sterile broth tubes, which should be in readiness. This should be done every twenty seconds for two or three minutes. The sub-cultures, duly labelled (and it is advisable to label them beforehand to save time), are to be incubated for two days at 37° C. It will be found that only the earliest sub-cultures grow. The organisms should be dead in one or, at most, two minutes. Carbolic acid kills Staphylococci almost as rapidly as it kills B. coli communis, and it does not make much difference whether they are suspended in broth or pure water. This experiment teaches that carbolic acid in the strength of 1 part in 40 is an extremely efficient disinfectant for *non-sporing* bacteria.

The experiment may be supplemented by similarly testing the powers of izal in a total strength of 1 in 400. Make a solution of 1 in 200 of "medical" izal, and mix it with an equal bulk of a broth culture or emulsion of Staphylococcus pyogenes aureus or B. coli communis. It will be found to kill quite as quickly as 1 in 20 carbolic acid. In both cases, lest it be supposed that enough of the disinfectant has been transferred by the platinum loop to the sterile broth to

restrain growth, re-inoculate one of the sub-cultures which has failed to grow from a fresh culture of the organism tested. Growth will readily occur, proving that the results were genuinely due to the death of the organisms.

(4) *Testing the germicidal Powers of Perchloride of Mercury upon non-sporing Bacteria.*—The most satisfactory organism for this purpose is B. coli communis, which fairly represents the average resisting powers of non-sporing organisms against mercuric salts; but in addition the disinfecting powers of the perchloride should be carefully tested upon Staphylococcus pyogenes aureus, which has very unusual powers of resistance against this salt. As in testing with carbolic acid, emulsions of the test organisms should be prepared from agar-agar cultures, and freed from lumps by filtration. A solution of perchloride of mercury in distilled water must be prepared, having a strength of exactly 1 in 500. As in the preceding experiment, a measured 5 cc. of this is to be mixed with a measured 5 cc. of the bacterial emulsion, and sub-cultures are then to be made by transferring a platinum loopful of the mixture to sterile broth tubes, which are then to be incubated at 37° C. for two days. (Experiment will show that there is no danger of transferring enough of the disinfectant to restrain growth in the sub-culture.)

In the case of B. coli communis, whether a broth culture or an emulsion in water be employed, sub-cultures must be inoculated as rapidly as possible after the mixture has been made, and it will be found that death has resulted in a minute or less.

In the case of Staphylococcus pyogenes aureus the

results are very different, and the experiment may be carried out in such a manner as to illustrate the influence of albuminous material in interfering with the disinfectant action of mercuric salts. To this end make two emulsions of the cocci, one in sterile distilled water and one in nutrient peptone broth. Filter them and measure out into two test-tubes 5 cc. of each. Add, to each, 5 cc. of the 1 in 500 perchloride of mercury solution, giving a total strength of 1 in 1000. Sub-cultures are to be made in broth in the usual way, but there is no need to begin to make them till ten minutes after mixing. After this they may be made at intervals of five minutes. Different strains of the coccus vary very widely in their resistance to mercuric salts, but it will probably be found that the watery emulsion will resist the action of the perchloride for fifteen to thirty minutes, or even longer, while the broth emulsion may survive even for forty-five minutes to an hour or more. The writer has met with one strain of the cocci which survived more than four hours under these conditions. In any case a very great difference will be found in the resistance of the cocci, according to the medium in which they have been exposed to the action of the perchloride. The broth emulsion will survive far longer than the watery emulsion, and it must be remembered that the broth more closely reproduces the conditions under which the disinfectant will be required to act in actual practice. An emulsion in serum would be found even more resistant than one in nutrient peptone broth, because it contains more albuminous material to neutralise the mercuric salt. The genuineness of the

experiment should be controlled by making an agar-agar sub-culture from the last broth culture which shows growth, to prove that the growth is truly the Staphylococcus aureus and not a chance contamination.

(5) *Testing the germicidal Powers of Mercuric Salts upon Bacterial Spores.*—Convenient spores for this purpose are those of the hay bacillus. They are more resistant than anthrax spores. A simple and easy method is that of Koch, in which the spores are dried on silk threads.* Cut up some plaited silk thread, such as is used for surgical ligatures, into lengths of half an inch or so. It is convenient to prepare thirty or forty threads at once. Put them in a Petri dish and sterilise them in the hot-air apparatus for an hour, taking care that the temperature does not rise much above 150° C. lest they become charred. Make a thick emulsion in sterile water from a sporing culture of B. subtilis (an agar-agar culture incubated for three days at 37° C. is suitable), and pour it over the threads in the dish. Let them soak in the emulsion for twenty minutes or so, and then, with sterile forceps, pick out the threads singly and lay them in a dry sterile Petri dish, which should then be covered up and put in the warm incubator at 37° C. to dry. Next day they will be ready for use.

This experiment may be arranged so as to demonstrate the difference in efficacy between a watery and an

* Although this method is a convenient one, it must be observed that it takes much longer to kill spores when thus prepared than when they are dried on the surface of smooth pebbles, or suspended as an emulsion in the disinfectant. This is probably because the poison does not so readily penetrate into the interstices of the thread.

alcoholic solution of a mercuric salt. Two wide-mouthed stoppered two-ounce bottles should have been cleaned and then sterilised, either by heat or by rinsing with pure nitric acid, and then repeatedly rinsing with boiled water. In the latter case, drain away the water and then put into one bottle a 1 in 1000 solution of perchloride of mercury in water, and into the other a 1 in 1000 solution of biniodide of mercury in methylated spirit. Put a dozen of the prepared threads into each bottle, and label the bottles with the date and the nature of the solution. The vitality of the spores may be tested after four to six hours, after twenty-four hours, and thereafter on successive days for a week or a fortnight. The testing of the vitality is to be carried out as follows: Put into a sterile covered capsule a solution of ammonium sulphide. Sterilise three beakers and fill them with boiled water. Take a thread from the disinfectant solution with sterile forceps, wash it gently in one of the beakers of water, and then put it into the ammonium sulphide for five minutes. (This precipitates the remaining traces of disinfectant in the thread as a black sulphide of mercury, insoluble and inert. Without this precaution the spores may be prevented from germinating. The ammonium sulphide itself exercises no appreciable disinfectant influence.) Next transfer the thread to two successive beakers of sterile water, washing it gently in each, and finally drop it into a sterile broth tube, or plant it on the surface of a sloped agar-agar tube, pressing it well into the medium with a sterilised platinum wire. Label the tube, and incubate at 37° C. for two days. Fresh ammonium sulphide and sterile

water ought to be used for each thread, lest chance spores washed off from the preceding thread should happen to lodge on the next and thus introduce a fallacy.

The experiment thus carried out will teach two very important lessons: (1) the extraordinary resistance of bacterial spores against even such powerful disinfectants as the mercuric salts, and (2) the relative inefficacy of alcoholic as against watery solutions. It will be found that the hay bacillus spores are alive in both solutions after six hours and after twenty-four hours. After forty-eight hours those in the watery solution of perchloride of mercury will probably be dead, but those in the spirit solution of biniodide of mercury will be alive at the end of a fortnight or more.

(6) *Testing the germicidal Powers of Carbolic Acid and Izal upon Bacterial Spores.*—These experiments can be carried out concurrently with the preceding, as they extend over a week or more. The details are almost identical. Solutions of carbolic acid (1 in 20) and of izal (1 in 200) are put into the sterile bottles, and the prepared threads, saturated with hay bacillus spores, kept in them for a week. The only difference in detail is that ammonium sulphide is not used in washing the threads; they are simply to be washed in two or three successive beakers of sterile water, to remove as much as possible of the disinfectant, before being planted in the culture-tubes. There is no occasion to test the vitality of the spores till the expiration of a week. Even then, or after many weeks, they will almost certainly be found alive. From this it will be learned that carbolic acid and izal, so powerful

in their action upon non-sporing bacteria, are practically of no use at all where spores are concerned.

At the discretion of the teacher, other disinfectants may be practically tested on lines similar to those indicated in the preceding experiments.

## TESTING GASEOUS DISINFECTION

It is possible to fit up in a laboratory an apparatus in which bacteria can be exposed to the action of any desired gas. Such experiments are however liable to mislead, for it is rarely possible to reproduce on a large scale, in a room, the high percentage of the disinfectant gas which is so easily attained in a small apparatus in the laboratory. For this reason it is much to be preferred that the tests should be carried out under the conditions which obtain in actual practice. In a hospital it frequently happens that a room has to be disinfected after an infectious case, and advantage may be taken of such opportunities. Away from a hospital, it may sometimes be possible to persuade a medical officer of health to permit tests to be carried out in any house in his district in which disinfection happens to be required. Failing this it is always possible to carry out the tests in any small empty room available, which must be specially prepared for the purpose.

The preparation of the room must be carefully attended to, as the success of aërial disinfection is chiefly dependent on the pains expended on sealing the room. All articles liable to be damaged by the gas employed (*e.g.*, in the case of sulphur or chlorine, all bright metal objects) must be removed from the room. All windows

must be closed, and the cracks sealed with paper pasted over them. The fire-place must be closed with boarding, and the cracks sealed with paper. Ventilators and any other apertures must be similarly closed, and every possible means of egress of the gas from the room obstructed as completely as possible. Paste and paper must be in readiness outside the door for sealing the cracks as soon as the disinfection has commenced. The floor should now be wetted, and the room rendered as damp as possible, since moisture is essential to the disinfectant action of the gases employed.

**Testing Disinfection with Sulphurous Acid Gas.**—The following organisms may be used in the experiments: Staphylococcus pyogenes aureus, Bacillus coli communis, and spores of the hay bacillus. They should be exposed under two conditions—quite superficially, and soaked into some porous material. Cultures of the organisms may be painted in a thin layer on pieces of sterile glass, or any impermeable material. Strips of sterilised linen or cloth should also be soaked in broth cultures or emulsions of the bacteria used. The success of the disinfection will be greater if the test organisms are exposed in a moist condition than if they are previously dried.

The room having been prepared, as above described, the infected test objects are labelled and placed in it in convenient positions. The cubic capacity of the room should have been calculated, and sulphur provided to the amount of two pounds for every 1000 cubic feet of space. The sulphur is placed in several iron pans in different parts of the room, and it is a safe precaution to place these pans, on iron tripods, in basins of water

much larger than the pans themselves, to avoid the risk of fire. The sulphur is now set on fire, and when it is clear that all the pans are burning well, the door is closed and pasted up with paper from the outside. It is left for twenty-four hours, and at the end of that time the door is opened. If the sealing of the room has been properly carried out, it should be only just possible to rush in and open the nearest window. When the air has cleared a little, the test objects should be transferred to sterile tubes or Petri dishes and taken to the laboratory. Cultures are then to be made from them, either by putting them bodily, if of suitable size, into sterile broth tubes, or by making emulsions or scrapings from them, with due aseptic precautions, and inoculating culture-tubes. It will probably be found that, while the non-sporing organisms, exposed superficially, have been killed, the hay bacillus spores are still living, while even the non-sporing organisms soaked into cloth or linen have escaped total destruction.

A similar experiment may be carried out with chlorine gas, and will yield results very little superior to those furnished by sulphurous acid, though wet spores, superficially exposed, may possibly be killed.

**Testing Disinfection with Formalin Vapour.**—The room should be prepared as in the experiment with sulphur, and strips of infected linen, glass, &c., similarly exposed. Since it has been claimed for formalin that it possesses greater powers of penetration than sulphurous acid gas, some of the test objects may be covered up with dusters or cloths. Various forms of apparatus for the production of formalin vapours are in use, and they vary in their efficacy. In the "Alfor-

mant lamp" the formalin is produced by heating tablets of paraform, and ten tablets are recommended for each thousand cubic feet of space to be disinfected. In the "Formogène Richard lamp" it is produced by the incomplete combustion of strong wood spirit. Kanthack found the latter more powerful and certain in its action than the Alformant lamp. The more recently introduced "glycoformal" apparatus of Lingner appears to be considerably more powerful than either, and in Klein's experiments gave far better results than sulphurous acid gas.

Cultivations are to be made from the test objects after exposure, as in the sulphur experiment. Superficially exposed non-sporing organisms should be killed with certainty, and, with the more powerful forms of apparatus, superficially exposed spores should be killed. It will be found, however, that even non-sporing organisms are apt to escape destruction if covered with a cloth, showing that even formalin has no great powers of penetration.

## THE TESTING OF FILTERS

**Filtration through Paper.**—Sterilise some stout filter paper in a hot air steriliser, taking care that the temperature does not rise so high as to char it (140° C. will be safe). Sterilise also a funnel and a beaker. Filter a few broth cultures of Staphylococcus pyogenes aureus, or B. coli communis, through the sterilised paper into the beaker. Note that the filtered fluid is quite turbid, but less so than the original cultures, because the larger clumps have been kept back, though isolated

bacteria can pass readily enough through the pores of the paper. Unless it is desired to determine the relative numbers of the micro-organisms, before and after filtration, there is no real need for sterilising the apparatus employed in this experiment.

**Testing an ordinary Domestic Filter.**—Procure a new filter of the carbon-block type. Pour into the receptacle water, to which has been added a broth culture of Bacillus prodigiosus, or some other easily recognisable organism not habitually present in water. Make cultivations from the filtrate, by spreading a loopful of it over the surface of sloped agar-agar tubes, or agar-agar plates. The cultivations should be incubated at 20° C. if B. prodigiosus has been used, as it forms no pigment at 37° C. The organisms will be found to have passed through the filter fairly readily, though a considerable proportion may be kept back.

If it be possible to procure an old domestic filter which has been in use for some months without having been sterilised, the following experiment may be performed: Take some ordinary water fresh from the tap, and with a graduated pipette add a quarter of a cubic centimetre of it to a sterile gelatin tube, melted at 30° C., mixing the two well together. Pour it into a sterile Petri dish and allow it to set. Now pass the water through the old filter, and make a similar plate cultivation with a quarter of a c.c. of the filtrate. Label the cultivations and incubate them at 20° C. for a few days, comparing them from day to day. It will be found that the number of colonies arising after filtration is greater, and probably very much greater, than in the original water.

**Testing a High-pressure Filter.**—This experiment can be carried out on a Pasteur or Berkefeld filter fixed in a house, or on a portable one in the laboratory.

The fixed house filter may be tested as follows: Supposing it to have been in use for a week or two without being sterilised, turn on the tap and let it run for a few minutes; then collect some of the water in a sterile test-tube, plug with cotton-wool, and convey to the laboratory. At the same time, collect some water in the same way from an ordinary tap on the same service. Make gelatin-plate cultivations from the two samples, using half a cubic centimetre of each water, and comparing the plates after three or four days' incubation at 20° C. It should be found that the number of organisms in the filtered water is considerably less than in the unfiltered, even though the filter has not been sterilised for a week or two.

Now sterilise the filter. Unscrew the parts and clean the outside of the filtering-candle with a brush to remove the layer of organic matter and bacteria which has accumulated on it. Then steam the candle in an autoclave, or suspend it, by strings from each extremity, in a large saucepan and boil it for half an hour. Screw the apparatus together again in its place, avoiding any contamination of the sterile candle, and turn on the tap. After the water has flowed for a few minutes, collect some as before in a sterile test-tube and add 5 cc. to 5 cc. of sterile broth in a sterile tube. Incubate at 20° C. for several days. No growth whatever should occur. Repeat the test after the filter has been in use for twenty-four hours: the filtrate should still be sterile,

but after two or three days bacteria will begin to appear in the filtrate.

A portable high-pressure filter can be tested in the laboratory in much the same way. The ordinary types of filter in laboratory use are provided either with an air pump, by which sufficient pressure is attained to drive the liquid quickly through the filter, or with an arrangement by which the receiver can be connected with a suction pump. The filtering candle is first sterilised in the autoclave, and the parts screwed together, with due attention to asepsis—*i.e.*, no unsterile thing must touch any part which will come into contact with the water after it has passed through the filtering candle. The receiving vessel should have been sterilised in the hot air steriliser. When all is ready, fill the upper part of the apparatus with water to which has been added a broth culture of Bacillus prodigiosus, and put the filter in action. Add 5 cc. of the filtrate to a sterile broth tube by means of a sterilised pipette, and incubate at 20° C. for several days. It should remain sterile. If growth occurs the filter was defective, or not properly put together after sterilisation. Test any growth which occurs by sub-culture on a sloped agar-agar tube incubated at 20° C. (In these experiments with water, the cultures should always be incubated at 20° C. because most of the common water organisms will not grow at 37°, and in the present experiment, the B. prodigiosus only forms its characteristic red pigment at the lower temperature.)

The test may be carried a stage further by pouring the sterile filtrate back into the receptacle and repeating the filtering. This should be done several times

on three or four successive days. B. prodigiosus will eventually appear in the filtrate, having grown through the pores of the filtering candle.

## TESTING SURGICAL DISINFECTION

**Testing the Disinfection of the Skin for Surgical Purposes.**—Boil two agar-agar tubes, and when melted pour out their contents into two sterile Petri dishes. Allow them to set firmly. Select for the experiment some person with ordinarily clean hands, but who has not just washed them. Let this person dab the fingers all over the surface of one of the agar-agar plates, firmly but lightly, so as not to break up the layer of agar-agar. Replace the cover and label the plate. Now let the subject of the experiment scrub the hands throughly for five or ten minutes with hot water, soap, and a sterile nail-brush, frequently rinsing them under a hot-water tap (the water from which is sterile). The hands are then to be dried with a sterilised towel, and the fingers dabbed as before over the surface of the second agar-agar plate. Label this and incubate both at 37° C. for two days. It will be found that a very abundant growth has occurred in the first plate: the colonies will be numerous and probably of several different sorts. Some of them may be examined microscopically: the prevailing sorts are commonly Staphylococci. The second plate will present a strong contrast to the first. Occasionally it will be found quite sterile, but in any case the number of colonies should be extremely small. From this experiment it may be learned that the thorough cleansing of the skin from its

surface layer of grease and dirt is the essential basis of surgical cleanliness, and is of far more consequence than the use of antiseptics.

Next let the hands be prepared as if for a surgical operation. Trim the nails and remove all loose and softened epidermis from beneath them and at their roots. Scrub as before with a sterile nail-brush, soap, and hot water for five or ten minutes, and then soak and scrub with a disinfectant solution (carbolic acid 1 in 20, izal 1 in 200, perchloride of mercury 1 in 1000 in water, or permanganate and hydrochloric acid solution). Finally, rinse in sterile water (holding them under the hot-water tap will suffice), lest any of the disinfectant be carried over to the cultures which are to be made and vitiate the experiment. A sterile agar-agar plate should have been poured out and allowed to set. Over the surface of this the fingers should be dabbed as in the preceding experiment. In addition to this scrapings should be made from beneath the nails and from about their roots with a sterile scalpel: the *débris* thus obtained should be transferred with a sterile platinum loop to sloped agar-agar tubes and distributed over the surface. Finally, snippings should be made from the surface of the skin with sharp sterile scissors, not so deeply as to draw blood, and should be transferred to broth tubes. All cultures, having been labelled, are to be incubated for two days at 37° C. The agar-agar plate, over which the fingers have been dabbed, should remain perfectly sterile, indicating complete surface disinfection. But in the majority of cases a few colonies will be found to have arisen from the material scraped from the nails, while it is not unusual for a few of the

skin snippings to yield growth. From this experiment it may be learned that surface disinfection of the skin, which is all that a surgeon really requires, is not difficult to attain, but that absolute sterility of the hands in all their crevices, and below the surface, cannot be certainly attained by any ordinary and practicable methods.

**Testing the Sterility of Objects used in Surgical Operations.**—It is sometimes a matter of importance to be assured of the sterility of such things as ligatures, sponges, dressings, and towels.

*Ligatures and Sponges* are habitually stored in a disinfectant solution after they have been rendered aseptic. In order to prove their sterility it is essential to get rid of the chemical disinfectant, which will otherwise restrain the growth of any living bacteria present. Portions must be removed with sterile scissors and forceps, and transferred, with due precautions, to beakers of sterile water, covered up with sterile glass plates. Should they have been stored in carbolic acid solution, it will suffice to soak them for five minutes in each of three successive beakers of sterile water. But should they have been stored in perchloride or biniodide of mercury solutions, this will not suffice. They must be placed, after a preliminary soaking in sterile water, in a solution of ammonium sulphide for five minutes, after which they must be again soaked in sterile water. Thus freed from every trace of the disinfectant, they are to be transferred to sterile broth tubes and incubated at 37° C. It is to be noted that all these proceedings involve a certain risk of chance contamination from the air, though but a slight one, if the manipulations be carried out with due care. Should

growth occur in any of the cultures, its exact nature must be determined by microscopic examination and sub-culture. The growth of a non-sporing organism from material which has been stored for any length of time in 1 in 20 carbolic or 1 in 1000 perchloride of mercury is, on the face of it, practically incredible; and in such a case accidental contamination during the manipulations might be very properly suspected.

*Dressings and Towels* may be tested by removing portions with aseptic instruments and transferring them directly to sterile broth tubes, with due precautions, and incubating at 37° C. Should the dressings be antiseptic ones, it will be needful to get rid of the antiseptic by a preliminary soaking in sterile water, with or without a soaking in a neutralising chemical substance, such as ammonium sulphide, according to the chemical nature of the antiseptic present.

## EXPERIMENTS IN MEDICAL DISINFECTION

**Testing the Effect of Disinfectant Gargles upon the Bacteria of the Mouth.**—This experiment, the details of which are due to Dr. Mervyn Gordon, is an instructive one because it teaches the enormous number of bacteria in the mouth, as well as the practical value of disinfectant gargles.

Take five small flasks holding about 200 cc. each. Into one put 99 cc. of water, and into each of the other four 90 cc. of water. Plug the necks with cotton-wool, heat them for half an hour in the steam steriliser, and then let them cool. Sterilise a watch glass, and collect

saliva from the mouth in it. Measure out, with a sterile pipette, exactly 1 cc. of the saliva, and add it to the flask containing 99 cc. Label this flask No. 1; it represents a dilution of 1 in 100. Shake it well for several minutes to ensure an equable distribution of the bacteria, and then, with a sterile 10 cc. pipette, measure out 10 cc. of its contents, and add this to the 90 cc. of water in flask No. 2, which then represents a dilution of 1 in 1000. After well shaking, transfer 10 cc. of this to the 90 cc. of water in flask No. 3, and repeat the process successively with flasks 4 and 5. Flask No. 5 will now contain a million-fold dilution of the saliva in sterile water, and each cc. of its contents will contain approximately the bacteria present in the millionth part of a cc. of the original saliva. Melt an agar-agar tube in boiling water and, when melted, cool it down to 45° C.: when it has reached this temperature, add to it 1 cc. from flask No. 5. Mix quickly, but carefully, and pour it out into a sterile Petri dish before it has begun to set, which will happen at 40° C.

Now gargle for four or five minutes with one of the following solutions: chlorine water, izal 1 in 200, or permanganate of potash 1 in 300. An hour after the gargling, taking no food in the meantime, collect saliva again in a sterile watch glass, and repeat the above experiment in every detail, using a second set of flasks and sterile pipettes. Label the two agar-agar plates, and incubate them (upside down, lest water condense on the lid and drip on to the surface of the agar-agar) for two days at 37° C. Then count the number of colonies in each plate. It will probably be found that, in the first plate, prepared from the natural mouth-

secretions, colonies have arisen to the number of about a hundred, more or less, indicating one million times that number in each cubic centimetre of the original saliva. (Even this figure is far below the truth, for large numbers of the mouth-bacteria will not grow in our culture media.) In the second plate, prepared from the saliva an hour after gargling, the number of colonies will be strikingly less, probably less than ten altogether. This teaches that the effect of a good disinfectant gargle persists for some little time.

**Testing the Dissemination of Bacteria from the Mouth in Talking.**—This experiment is unsuited for performance by or before a class, because it demands a quiet room, free from draughts or disturbance. It may be carried out in private by the teacher, and the results demonstrated to the class. A dozen or more sterile agar-agar plates are prepared. These are exposed in various places about the selected room at measured distances from the point at which the experimenter is to stand. He then retires to an adjacent room, and rinses his mouth thoroughly with a watery emulsion made from a brightly-coloured agar-agar culture of Bacillus prodigiosus. Returning stealthily to the prepared room, he takes up his position and reads aloud, or delivers an oration for half an hour. The louder he talks, and the more he mouths his words, the better for the success of the experiment: quiet talking will produce little effect. The plate cultures are then covered up, labelled, and incubated for four or five days at 20° C. They will be found to have been infected, as shown by the crimson colonies of the B. prodigiosus which arise, in varying degree according

to their situation, and to the air currents in the room; they may readily be infected up to a distance of 20 feet from the speaker.

**Demonstration of the Infective Agent in a Specific Fever.**—There is an educational value in actually seeing the pathogenic organisms from a known case of infective disease, since the concrete nature of infection is thus impressed upon the learner. Diphtheria is well suited for such a demonstration, and if a case of the disease be accessible, the teacher should prepare cultures, on serum or serum agar-agar, and demonstrate them, after incubation, to the class. Microscopic preparations from the cultures should be shown. If the teacher and the building are licensed for inoculation experiments, the demonstration will gain in force by inoculation of a guinea-pig with a pure culture of the diphtheria bacillus derived from a known case of the disease. The abundance of the bacilli in the mouth-secretions may be demonstrated by collecting the saliva from a case of diphtheria, and carrying out a series of dilutions, as described above, under the heading of "testing disinfectant gargles." A ten thousand-fold, or even one hundred thousand-fold dilution of the tonsillar exudation should yield, on serum, numerous colonies of diphtheria bacilli.

**Testing the Disinfection of the Sputum.**—The more tenacious the sputum, the more difficult is it to disinfect by chemical means, because it does not mix well with the disinfectant. In the case of such disinfectants as perchloride of mercury or carbolic acid, an envelope of coagulated material forms around the masses of sputum and hinders the access of the disin-

fecting agent to their interior. This is especially the case with the "nummular" sputa of phthisis. The disinfection of such sputa, as regards tubercle bacilli, can only be tested satisfactorily by inoculation experiments upon animals. As a class experiment, the following test is instructive:

Procure some sputum of tenacious consistence, and place portions of it in two sterile Petri dishes. Over one portion pour a considerable excess of 1 in 20 carbolic acid solution. Over the other pour a similar excess of a solution of "chloride of lime" (bleaching powder). The solution should be made by shaking about two grammes of fresh chloride of lime in 100 cc. of distilled water: it will only partially dissolve. Compare the two specimens at the end of an hour or so. In the carbolic acid the sputum will be white and coagulated: if the mass be a large one, a portion removed from its interior may still yield a growth of cocci from its central part on culture. (Experiment has shown that virulent tubercle bacilli may still remain alive in the central portions on the "nummular" sputa of phthisis even after twenty-four hours in 1 in 20 carbolic, or in 1 in 500 perchloride of mercury.) But in the sputum exposed to chloride of lime solution a very different condition will be revealed. It will be found completely broken up and dissolved, so that the disinfectant has been able to act upon every portion. A culture in broth will be found quite sterile.

The disinfection of fæces or other excreta may be tested on similar lines.

* * * * *

The foregoing practical experiments, personally

carried out by the learner, will be sufficient to carry conviction as to the cardinal facts of disinfection set forth in this book. The statements contained in the two closing lessons may, to a large extent, be illustrated by the teacher by means of microscopic preparations and cultures of the specific organisms described.

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